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SOME OBSERVATIONS ON INFECTION OF THE BOVINE UDDER WITH *MYCOBACTERIUM TUBERCULOSIS* AND *CORYNEBACTERIUM PYOGENES*

By

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INTRODUCTION

The experience of veterinary workers in the field of public health has led them to believe, on the basis of biological examination of milk samples, that only cows with tuberculous udders yield infected milk and that when these animals are removed the milk is free from tubercle bacilli, even although 30 to 40 per cent. of the cows in a herd are tuberculous. When searching tests are employed, however, milk from tuberculous cows sometimes contains small numbers of bacilli. This fact could be explained by the presence of a quiescent lesion in the udder from which a few bacilli sometimes escaped or an occasional bacteraemia, because experiments indicate that the normal udder will permit the escape of tubercle bacilli from the blood into the milk. The subject has been reviewed by Francis (1947), but as no recent work has been carried out some observations were made in a herd from which milk samples were being taken for the control of streptococcal mastitis (Francis, 1949). This was referred to as herd 1.

The guinea-pig is not usually used in research on *C. pyogenes* infections in this country, although it was shown by Carré (1912) and Weinberg, Forgeot and Richart (1938) that the organism would infect guinea-pigs. The finding of abscesses containing large numbers of *C. pyogenes* in the guinea-pigs inoculated with milk was an incidental finding of some interest in this investigation, especially as the organism had not been isolated by the ordinary bacteriological techniques.

METHODS

Approximately 1·5 ml. amounts of milk from each quarter of four (or occasionally five) cows were mixed and 10 ml. injected intraperitoneally into two guinea-pigs, i.e., 1·25 ml. of milk from each quarter was subject to biological test. In addition, 0·01 ml. of milk from each quarter was streaked on an aesculin blood-agar plate (Francis, 1948) and a loopful of milk was again streaked out after 18 hours' incubation.

RESULTS

Mycobact. tuberculosis

The herd consisted of 18 milking cows and the results of the examination are shown in Table 1. All four cows in group 1 were tuberculin positive and two in each of the other three groups. Groups 3 and 4 are not shown in the table because no positive

TABLE I

RESULTS OF INOCULATING GUINEA-PIGS WITH MILK FROM GROUPS OF COWS[1].

Date	*Group 1*			*Group 2*		
	Time[2] to death in weeks	*Presence of pathogens*		*Time to death in weeks*	*Presence of pathogens*	
		T.B.[3]	*C.P.*		*T.B.*	*C.P.*
31.5.45.	K.11 K.11	— —	— —	K.11 K.11	—	—
20.6.45.	K.30 D.3	— —	— +	K.30 D.6	+ —	— +
12.7.45.	K.31 D.4	— —	— —	K.31 D.3	— —	— +
27.7.45.	K.30 D.14	— —	— —	K.30 D.3	— —	— +
22.8.45.	K.36 D.10	— —	— —	K.36 K.36	— —	— +
6.9.45.	K.34 K.34	— —	— —	K.34 D.3	— —	— +
18.9.45.	K.3 D.7	— —	— —	K.32 K.32	— —	— —
23.10.45[4]	K.27 K.27	+ —	— —	K.27 K.27	— —	— —

[1] There were four or sometimes five cows in each group. Groups 3 and 4 are not shown in the table because no positive results were obtained in animals inoculated with their milk. All four cows in group 1 were tuberculin positive and two in each of the other groups.

[2] K = Killed. D = Died.

[3] T.B. = Tubercle bacilli. C.P. = *C. pyogenes*.

[4] Cow 12 was included in all but the last test of group 2.

findings were obtained. It will be seen that a guinea-pig inoculated with milk from group 2 on 20th June, 1945, and one inoculated with milk from group 1 on 23rd October, 1945, became tuberculous. The first tuberculous guinea-pig was killed seven months after inoculation, and it is of interest that although the stomach and

spleen were adherent to each other and to the abdominal wall (it is possible that this was due to an old infection with *C. pyogenes*) there were only a few lesions of tuberculosis in the spleen, lungs and bronchial lymph-nodes. As smears from the bronchial lymph-nodes showed a few acid-fast bacilli, a suspension was treated with 5 per cent. caustic soda and inoculated onto tubes of Dorset's egg medium with and without glycerol. Growth was much better in the tubes containing no glycerol. The remainder of the suspension was inoculated into the thigh of two guinea-pigs, which died with extensive lesions of tuberculosis two and three months afterwards. One concludes that the organism recovered was a virulent strain of the bovine type, but was present in the milk in very small numbers.

The second guinea-pig which became tuberculous following the inoculation of milk was also killed seven months after inoculation and again contained only slight lesions of tuberculosis. Acid-fast organisms were found and no further examination was carried out.

Corynebacterium pyogenes.

C. pyogenes was first observed in two guinea-pigs which died three and six weeks after inoculation of milk from groups 1 and 2 on the 20th June, 1945 (Table 1). These guinea-pigs had adhesions in the peritoneal cavity and small abscesses in the liver. There were extensive necrotic and purulent lesions in the lung, together with pleurisy and pericarditis. Films from the peritoneal lesions, lung and pleura revealed numerous diphtheroid bacilli and pure cultures of small haemolytic colonies were obtained from the lung, pleura and heart blood. These organisms had exactly the same cultural properties on blood agar plates, solid serum and in litmus milk as strains of *C. pyogenes* isolated from cases of mastitis. It will be seen that three other guinea-pigs inoculated with milk from group 2 died and abscesses containing *C. pyogenes* were found in one other. In the two guinea-pigs which died three and eight weeks after the inoculation of milk on 22nd July, 1945, and 6th September, 1945, there were extensive lesions in the abdominal cavity but none in the thoracic cavity. One of the guinea-pigs which were killed thirty-one weeks after inoculation of milk on 22nd August, 1945, had a large abscess in the liver which contained numerous *C. pyogenes*.

These findings are rather surprising in view of the fact that *C. pyogenes* was not obtained by cultural methods from the milk used to inoculate guinea-pigs, but the subsequent history of cow 12 in group 2 (Table 2) which eventually developed "summer" mastitis is of interest. It will be seen that the first lesion in this cow was a small, hard subcutaneous swelling in the posterior surface of the quarter. It is of interest that Mr. W. F. Morton saw two cows with similar lesions which subsequently developed "summer" mastitis during 1950.

TABLE II

CLINICAL HISTORY OF COW 12.

Date	*History*	*Presence of Pathogens*	
		LH Quarter	*RH Quarter*
4.11.45.	Cow going dry. All quarters normal.	0	0
26.3.46.	Cow dry. All quarters normal.	No sample could be obtained	0
10.4.46.	A very hard subcutaneous easily movable swelling was palpated on the posterior surface of quarter C.	—*	—
15.4.46.	Whole of quarter C swollen but the small firm swelling could still be detected. 500 ml. odourless yellow green pus obtained.	*C. pyogenes* * +++	0
16.4.46.	No change in quarter C. 50,000 units of penicillin injected into all quarters. Very little change during the next 3 days.	—	—
23.4.46.	Thick yellow secretion persisting in quarter C. 0·5 ml. of phenanthraquinone in 25 ml. liquid paraffin injected.	—	—
8.5.46.	Quarter C now very firm, yellow secretion containing large yellow flakes.	*C. pyogenes* * +++	0
18.6.46.	Quarter C small and firm and secretion watery with a few clots. 0·5 gm. phenanthraquinone injected into quarters A and B.	*C. pyogenes* * +++	*C. pyogenes* +
9.7.46.	Quarter C firm and contained yellow fluid with a white sediment. Quarter B— yellow fluid and slimy clots.	*C. pyogenes* * +++	*C. pyogenes* * +++
18.7.46.	Cow had calved three days previously and was giving normal milk in quarters A and D, but thick yellow curds in B and C.	*C. pyogenes* * +++	*C. pyogenes* * +++

*—indicates that no bacteriological examination was made.

The demonstration of *C. pyogenes* in the milk by the inoculation of guinea-pigs, together with the subsequent history of cow 12, indicate that *C. pyogenes* may be present in the udder for a considerable period before the onset of "summer" mastitis. The fact that *C. pyogenes* may be present in the udder, without causing obvious disease, is supported by the following observations on cow 108. The cow was dry on 18th April, 1946: the udder contained a honey-like secretion and no pathogens were isolated. The cow was examined again on 13th June, 1946, and 28th June, 1946, when the right fore-quarter contained a clotted, slightly blood-stained fluid and *C. pyogenes* was isolated in small numbers. The quarter was not swollen and "summer" mastitis did not develop. The cow calved on 3rd July, 1946, she milked normally and *C. pyogenes* was not isolated again at seven subsequent examinations.

The view that the udder may be "latently" infected with *C. pyogenes* and the onset of mastitis precipitated by another cause gives some support for events following injection of two cows with phenanthraquinone. Phenanthraquinone is 9 to 27 times more potent as a bacteriostatic agent against streptococci than proflavine. Like proflavine, however, it has no chemotherapeutic action when injected into the yolk-sac of the chick-embryo (Francis, 1946). It is also interesting that although it had such good bacteriostatic action it had very little bactericidal action. Thus, 1/10,000 proflavine killed various mastitis streptococci in an average of 1·2 hours, but the same concentration of phenanthraquinone did not do so before 58·8 hours, and 1/100 phenanthraquinone took 28·7 hours to kill. Nevertheless, it was thought that phenanthraquinone might persist in the udder and prevent the development of "summer" mastitis. In all, eight dry cows were injected during the summer.

On 8th May, 1946, cow 19 was springing to calve and the right hind quarter was slightly swollen, but *C. pyogenes* was not isolated. The quarter was injected with 2 g. of phenanthraquinone in liquid paraffin. The cow calved on 29th May, 1946, and only a little watery secretion was obtained from this quarter. On 30th May, 1946, *C. pyogenes* was isolated in large numbers. The two fore-quarters of cow 113 were injected with 0·5 g. of phenanthraquinone in liquid paraffin on 13th June, 1946, when the secretion was free from *C. pyogenes*. On 18th July, 1946, the cow had typical "summer" mastitis in the left fore-quarter and *C. pyogenes* was isolated in large numbers.

These results may have been coincidence, but "summer" mastitis was not severe at the time and it is believed that the irritant action of the drug rendered the quarters more susceptible.

Further observations on Infection of Guinea-Pigs

Two guinea-pigs were inoculated intraperitoneally with pus from a submaxillary abscess in a cow which was suspected of being

tuberculous on clinical grounds. Diphtheroid bacilli but no acid-fast bacilli were observed in smears. One of the guinea-pigs died four days later; there were pneumonic lesions in the lung from which a pure culture of *C. pyogenes* was obtained. A 48-hour blood-broth culture of *C. pyogenes* obtained from the guinea-pig was inoculated into both lungs of four guinea-pigs (1-4). All guinea-pigs appeared normal the next day. One died five days later with typical pneumonia and pleurisy and a large amount of blood-stained pleural fluid. Two more died on the 10th day with similar lesions, but the remaining guinea-pig survived.

The peritoneal fluid from the guinea-pig dying on the fifth day was inoculated in amounts of 0·75 ml. into each lung of three guinea-pigs (5, 6, 7) and three rabbits. The guinea-pigs died five, seven and eleven days later with lesions of pleurisy and pneumonia. One rabbit died nine days later with a purulent pneumonia and pleurisy. The other rabbit died fifty days after inoculation, and it showed fibrous peritonitis and large amounts of green turbid peritoneal exudate. *C. pyogenes* was seen in smears made from lesions of both rabbits.

Three small guinea-pigs (8, 9, 10) were inoculated into each lung with 0·5 ml. of pleural fluid from guinea-pig 5. They were all dead the following morning and yielded about 7 ml. of blood-stained pleural fluid containing numerous *C. pyogenes;* the pooled material was used to inoculate five more guinea-pigs. Two (11 and 12) which were inoculated with 0·5 ml. into each lung died the following day and one inoculated in the same way (13) died after two days and all showed typical pleurisy. Two were inoculated intraperitoneally with 0·5 ml. of material; one (14) died the following day with serofibrinous peritonitis and one (15) survived for six months.

Pleural fluid from guinea-pigs 11 and 12 was used to inoculate two more guinea-pigs (16 and 17) with 0·5 ml. into each lung. They died the following day with typical lesions and the fluid from them was used to inoculate two guinea-pigs (18 and 19) in the same way. No. 18 died one and No. 19 three days later. A culture from guinea-pig 18 was inoculated in 0·5 ml. amounts into each lung of two guinea-pigs. One died four days later with typical lesions and one survived for seven months. There was therefore no evidence that this series of passages had increased the virulence of *C. pyogenes* for the guinea-pig.

DISCUSSION

The finding of an occasional milk sample slightly infected with tuberculosis in an infected herd when there was probably no tuberculosis disease of the udder is in accord with the results obtained by Mohler (1903; see Francis, 1947). As the samples were taken carefully from individual quarters, it is assumed that tubercle bacilli were present in the milk. The presence of these

bacilli could be due to a lesion in the udder from which a few bacilli were occasionally excreted. There is other evidence, however (Francis, 1947), that tuberculous cows occasionally have tubercle bacilli in the blood stream and that such bacilli can pass into the milk. This is thought to be the explanation of the present findings.

The occasional isolation of *C. pyogenes* indicates that, as with all other organisms causing mastitis, there may be a latent infection of the udder and the value of penicillin in the prophylaxis of "summer" mastitis (Pearson, 1950) may be partly due to the cure of these latent infections. On the other hand, it appears that a slightly irritant substance with little or no real therapeutic value may precipitate the onset of "summer" mastitis. These latent infections could be established by the entry of organisms via the teat canal, but it is known that a high proportion of cows harbour *C. pyogenes* in the tonsils or vagina (Francis, 1941) and bacteraemia must occur quite frequently in order to produce the internal abscesses infected with *C. pyogenes* which are known to be common in the bovine. As with tubercle bacilli these organisms in the blood stream may pass into the milk and set up mastitis or a latent infection of the udder.

SUMMARY

A small herd was divided into groups of four cows each and pooled samples of milk from each group were examined on eight occasions. Two guinea-pigs were each injected intraperitoneally with 10 ml. from every sample of milk, i.e., approximately 1·25 ml. of milk from each quarter was examined on each occasion. Two guinea-pigs developed very slight tuberculosis.

Five guinea-pigs inoculated with the milk died and showed lesions from which pure cultures of *C. pyogenes* were isolated. An abscess containing *C. pyogenes* was found in one other guinea-pig. All but one of the infected guinea-pigs were injected with milk from group 2 and one cow in this group subsequently developed a typical infection of *C. pyogenes* in the udder. *C. pyogenes* was isolated from the udder of a cow in another herd that developed no obvious disease.

It is suggested that both tubercle bacilli and *C. pyogenes* may occasionally pass from the blood stream into the milk. They may then be eliminated, or give rise to subclinical or clinical infections.

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EXPERIMENTAL PARATYPHOID INFECTION IN PIGS

By

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INTRODUCTION

Necrotic enteritis caused by *Salmonella suipestifer* is a serious disease of young pigs which, by death of the animals or reduced efficiency of food conversion, causes heavy loss to pig farmers. For many years it was believed that the disease could not exist independently but was always associated with swine fever.

One of the principal causes for this belief was Salmon's work in which he attempted to prove that hog cholera was a bacterial disease. Salmon (1889) was able to produce experimentally a bacterial disease similar in many ways to hog cholera, but the disease did not spread naturally and easily to susceptible pigs. When it was discovered that hog cholera was a virus disease the fact that Salmon did produce a true bacterial disease was forgotten. As recently as 1933, M'Fadyean criticised severely the investigations of Biester, Murray, Purwin and McNutt (1927) in America and of Hindmarsh and Edgar (1933) in Australia on the bacterial disease. There is now no doubt that a salmonellosis of young pigs, usually called necrotic enteritis because of the typical lesions, does exist as an independent disease.

Much has been written on necrotic enteritis but little systematic experimental work has been done. Dammann and Stedefeder (1910) made extensive experiments on swine fever and *S. suipestifer* infection, but it is clear from the details given that many of the successful *S. suipestifer* infections were actually accidental swine fever, since the interval between the infection with *S. suipestifer* and the onset of disease was too long. The dangers of this work were clearly shown by Doyle and Walkey (1946). They showed that viscera from typical outbreaks of necrotic enteritis caused disease and death only in pigs not immunised against swine fever. The most complete work on the disease yet published was done by Murray, Biester, Purwin and McNutt (1927). They infected pigs artificially with *S. suipestifer* and recorded the results in great detail. The doses of culture were, however, very large and probably the lesions were as much due to direct toxic action of the culture as to true infection. In Northern Ireland, Shanks and Lamont (1938) were able to reproduce the disease to a small extent by feeding large amounts of culture. Hegyeli (1942) showed that bacteria-free filtrates of *S. suipestifer* cultures were very toxic when given intravenously. The filtrates had no effect on rabbits, guinea pigs, mice and pigeons. A curious fact was that pigs actively immunised against swine fever were less susceptible to the toxin. A possible explanation, not mentioned by Hegyeli, is that during immunisation pigs often go through a mild *S. suipestifer* infection and the dangers of immunising pigs in poor condition are well known. Kauffmann (1941) has pointed out that salmonella of various types including *S. suipestifer,* are found in the lymph glands of apparently normal animals including man. By taking bulk samples of lymph glands from groups of 25 pigs Rubin, Sherago and Weaver (1942) found 242 strains of salmonella

in 47·5 per cent. of 45 lots: five out of 50 pigs examined individually were positive. Bruner and Moran (1949) reported similar findings.

Gwatkin and Moynihan (1944) reviewed published work on the disease and described work of their own designed to clear up some of the confusion. They varied the rations and also employed intestinal irritants, but with little effect on the incidence of infection in spite of the large doses of culture which were used. In a later paper they tried 14 different strains of *S. suipestifer* and necrotic bowel with little success and concluded that the concept of *S. suipestifer* as a primary cause of the disease needed revision.

EXPERIMENTAL WORK

It is clear from the literature there is a wide-spread belief that errors in feeding and management play an important part in the onset of the disease. Various factors such as a vitamin imbalance and iron deficiencies have been blamed. At one time, a lack of nicotinic acid was regarded as primarily responsible for the disease. The author, in collaboration with another institute, tested the resistance of two groups of pigs to *S. suipestifer* infection. These pigs, one group of which was anaemic and undersized, were specially reared for the experiment. The results were somewhat startling in that the anaemic pigs were much more resistant to infection than their well grown companions. The initial experiments of the present series were therefore designed to show that pigs were susceptible to infection in a natural way when kept free from every known nutritional defect, fed on a good ration containing yeast, and maintained under the system of management—completely open air with free range—generally considered best. Pigs under these conditions were easily infected by lightly spraying the grass of the paddock in which they were kept with a broth culture of *S. suipestifer*. The condition produced was apparently identical with the natural disease.

When it was proved that normal pigs could be infected easily, it was decided to find a suitable dose of culture to test the effect of nutritional and other factors. The amount of infection picked up at pasture was likely to be variable. Previous work had shown that 5 cc. per os. would kill the majority of the pigs. As it is unusual to find a mortality of this order in field outbreaks the doses used in these experiments were chosen in an attempt to set up a condition similar to that commonly found in the field.

For each experiment the available pigs were grouped, the litters being equally divided between the groups to give even litter distribution and if possible equal average weight per group. It was originally intended to use 10 pigs per group but in some of the later experiments only five to eight pigs per group were available. These pigs, which were normal in every way, were bred specially for the purpose on a separate farm where they were not exposed at any time to salmonella infection. To check their freedom from infection the faeces of the stock pigs were tested weekly for salmonella on the selective medium devised for the

purpose (Slavin, 1943); they were invariably negative. As further evidence of the absence of natural infection, it may be added that no pig dying from natural causes was ever found infected with salmonella. On several occasions blood samples, taken from each pig, were negative. Pigs later used for swine fever work were not only free from salmonella infection at death, but showed an unusual resistance to secondary infections in experimental swine fever.

The pigs were brought to the laboratory one to two days before the experiment was due to begin, starved 24 to 36 hours and then fed individually with the dose of culture in a little food. The uninfected controls were starved in the same way. The doses used were 0·5 cc., 0·05 cc., 0·005 cc., and 0·0005 cc., of an 18-hour peptone broth culture set to MacFarland Tube 1 opacity. The dilutions were made in peptone broth and for feeding to the pigs each dose was made up to approximately 5 cc., in the same medium. Dilutions were also made and plates poured to estimate the numbers of living organisms. The preparation of the doses, pouring of the plates and the feeding of the pigs were done as quickly as possible to avoid multiplication of the organisms; moreover, during hot weather the broth was chilled. The strain of *S. suipestifer* used throughout came from an extensive outbreak of uncomplicated paratyphoid on a large pig farm and was kept dried to maintain uniform virulence. The organism was plated each time it was used and a smooth colony was selected.

The pigs were weighed weekly for a total of eight weeks. The average weight gains per group of the infected groups were compared with the weight gains of the uninfected control group, the controls being taken as 100. The average gains are those of the survivors. The results expressed in this way are shown in the upper part and the individual weights in the lower part of the tables.

Experiment 1

Five litters varying from 64 to 71 days in age were divided into four groups of ten pigs each and placed in grass pens about 50 yards apart. Each pen was provided with a wooden pig house. Table I shows the average weight per group at the beginning of the experiment, the number of organisms given as calculated from plate counts and the gains in weight over a period of eight weeks. A blank in a column indicates that there was no change in the weight gain. A cross indicates the death of a pig.

Results. The first pigs to show signs of infection were in Group 1 (0·5 cc.) after 72 hours. The symptoms were failure to feed and profuse diarrhoea. Group 2 (0·05 cc.) was not feeding well but did not have diarrhoea. The control group was normal. At 96 hours all the infected pigs were off feed and all had diarrhoea except group 2. The diarrhoea gradually disappeared. The lesions in the dead pigs were extensive and characteristic.

TABLE I

AVERAGE WEIGHT GAINS OF INFECTED GROUPS EXPRESSED AS PERCENTAGE OF WEIGHT GAINS OF UNINFECTED CONTROLS

Group	*Dose*	*Average weight in lb.*	*Weeks after infection*							
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1	0·5 cc. 383×10^6	37·6	12·37	17·48	25·14	26·19	33·21	43·67	44·59	50·92
2	0·05 cc. $38·3 \times 10^6$	36·4	13·40	—	20·11	31·90	41·78	45·18	51·08	52·31
3	0·005 cc. $3·83 \times 10^6$	38·8	36·08	37·14	58·66	59·52	61·78	61·44	64·32	65·28

Dose and Group	*Number of Pigs*	*Weight in lb. at beginning of experiment*	*Weeks after infection*							
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Group I 0·5 cc. 383×10^6	1025	35	37	34	32	41	42	48	49	54
	1026	27	24	+	—	—	—	—	—	—
	1035	35	33	33	39	39	45	50	52	57
	1036	32	36	33	32	28	30	33	33	34
	1045	36	38	41	46	47	53	59	62	70
	1046	46	43	51	47	46	50	53	56	63
	1054	47	49	38	+	—	—	—	—	—
	1055	50	52	52	62	64	67	74	78	84
	1060	33	37	33	37	37	41	48	49	55
	1061	35	+	—	—	—	—	—	—	—
Group II 0·05 cc. $38·3 \times 10^6$	1030	36	36	36	44	41	46	50	56	60
	1031	25	37	34	40	38	45	45	47	50
	1041	41	34	34	36	39	44	48	52	55
	1042	40	40	38	46	46	48	51	53	57
	1043	42	43	46	49	49	58	61	67	68
	1051	40	44	44	52	52	57	61	65	71
	1052	38	36	37	37	42	46	51	54	59
	1053	36	36	26	22	+	—	—	—	—
	1066	34	38	33	38	42	45	47	50	54
	1067	32	33	32	36	39	44	49	54	57
Group III 0·005 cc. $3·83 \times 10^6$	1027	22	26	25	31	30	31	34	37	41
	1037	36	38	42	44	45	49	53	56	59
	1038	45	42	42	47	45	48	50	51	57
	1047	44	49	52	62	65	72	78	82	87
	1048	40	46	43	54	66	61	68	72	78
	1056	45	54	51	59	62	69	72	77	80
	1057	46	50	51	57	58	65	71	74	80
	1062	33	34	37	41	40	44	47	50	53
	1063	35	38	41	49	51	66	60	64	68
	1068	42	46	+	—	—	—	—	—	—
Group IV Controls No Infection	1028	28	41	42	49	52	59	64	68	75
	1029	40	51	54	62	65	71	78	82	90
	1039	32	40	37	42	40	45	50	50	56
	1040	37	49	50	56	64	72	77	82	88
	1049	32	43	47	55	57	65	69	72	76
	1050	36	42	44	54	56	63	69	72	75
	1058	43	52	46	57	61	70	73	78	84
	1059	46	61	64	77	82	91	99	107	116
	1064	39	+	—	—	—	—	—	—	—
	1065	37	42	42	42	45	49	53	55	62

One pig with control group suffered from severe fits and was found dead. It was free from macroscopic lesion and bacteriologically negative.

— = weight gain was negative.

+ = pig died.

TABLE II

AVERAGE WEIGHT GAINS OF INFECTED GROUPS EXPRESSED AS PERCENTAGE OF WEIGHT GAINS OF UNINFECTED CONTROLS

Group	*Dose*	*Average weight in lb.*	*Weeks after infection*							
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1	0·5 cc. 202×10^6	43·6	—	13·89	53·49	58·06	65·79	NOT WEIGHED	79·59	93·44
2	0·05 cc. $20·2 \times 10^6$	40·4	86·67	69·44	86·05	77·42	92·11		108·16	94·52
3	0·005 cc. $2·02 \times 10^6$	42·4	66·67	55·56	88·37	70·97	89·47		103·06	83·56

Dose and Group	*Number of pigs*	*Weight in lb. at beginning of experiment*	*Weeks after infection*							
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Group I 0·5 cc. 202×10^6	1090	45	45	47	49	53	58	NOT WEIGHED	67	76
	1096	51	51	49	55	58	60		65	71
	1116	41	42	44	48	51	53		58	67
	1122	39	38	41	44	46	49		54	61
	1140	42	40	42	45	46	48		52	57
Group II 0·05 cc. $20·2 \times 10^6$	1092	42	45	48	50	51	53		61	67
	1098	47	50	54	58	60	65		72	78
	1115	41	43	46	48	51	56		62	70
	1121	45	50	53	56	60	66		74	82
	1120	27	27	26	27	28	32		39	43
Group III 0·005 cc. $2·02 \times 10^6$	1089	37	38	39	43	44	47		52	55
	1113	49	59	57	63	65	71		78	80
	1118	40	42	44	45	46	50		58	63
	1114	39	43	44	47	48	53		59	65
	1119	47	46	48	52	53	59		66	71
Group IV Controls No infection	1093	37	39	41	43	46	47		56	61
	1099	53	57	64	66	73	75		86	95
	1104	41	44	48	49	52	57		62	68
	1117	30	33	37	37	40	42		46	51
	1123	37	40	44	46	49	53		61	69

Experiment 2

In this experiment in which older pigs were used there were 20 animals from six litters from 65 to 81 days old. During the whole course of the experiment they were kept in adjacent brick sties with concrete floors which were brushed out and cleaned down every day. The pigs were infected in the same way as those in Experiment 1. The first signs of infection as indicated by the appearance of anorexia and diarrhoea occurred at about the same time as in Experiment 1. The results are shown in Table II.

TABLE III

AVERAGE WEIGHT GAINS OF INFECTED GROUPS EXPRESSED AS PERCENTAGE OF WEIGHT GAINS OF UNINFECTED CONTROLS

Group	*Dose*	*Average weight in lb.*	*Weeks after infection*							
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1	0·05 cc. $17·2 \times 10^6$	26·5	—	—	12·24	27·65	29·74	30·28	36·53	36·27
2	0·005 cc. $1·72 \times 10^6$	29·0	35·41	45·45	69·38	67·37	70·88	71·15	74·90	77·28

Dose and Group	*No. of Pigs*	*Weight in lb. at beginning of experiment*	*Weeks after infection*							
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Group I 0·05 cc. 172×10^6	1204	20	20	20	24	20	20	20	24	24
	1205	24	23	22	24	26	27	29	33	35
	1194	30	30	32	37	41	43	46	52	54
	1195	27	27	30	29	30	30	32	34	35
	1197	34	34	33	36	35	36	37	39	38
	1196	24	23	20	16	+	—	—	—	—
Group II 0·005 cc. $17·2 \times 10^6$	1191	36	37	41	45	51	54	60	65	71
	1193	34	38	42	46	48	52	56	62	65
	1192	37	39	41	43	46	49	50	57	59
	1203	23	25	25	29	28	29	31	37	38
	1202	20	20	20	22	22	21	24	26	27
	1204	24	25	29	30	36	36	42	49	51
Group III Controls No infection	1190	37	42	44	46	48	49	55	61	63
	1199	25	29	33	32	37	39	43	49	50
	1198	23	28	30	29	32	32	38	43	46
	1119	33	38	45	46	49	50	56	62	65
	1128	37	43	49	51	58	62	66	74	75
	1200	26	30	33	36	42	44	48	55	59

TABLE IV

AVERAGE WEIGHT GAINS OF INFECTED GROUPS EXPRESSED AS A PERCENTAGE OF WEIGHT GAINS OF UNINFECTED CONTROLS.

Group		*Dose*	*Average weight in lb.*	*Weeks after infection* 1	2	3	4	5	6	7	8
12 weeks	1	0·5 cc. 169×10^6	34·2	—	12·24	20·00	31·91	40·32	55·84	56·31	59·7
	2	0·05 cc. $16·9\times10^6$	34·2	111·11	61·22	61·33	72·34	70·16	84·41	75·26	74·2
8 weeks	3	0·05 cc. $16·9\times10^6$	34·8	89·47	31·25	53·12	68·88	74·78	79·86	78·03	88·2
12 weeks	4	0·005 cc. $1·69\times10^6$	34·4	111·11	79·59	74·66	89·36	89·51	96·75	92·10	92·3
8 weeks	5	0·005 cc. $1·69\times10^6$	34·6	78·94	70·83	81·25	78·88	90·43	89·26	87·86	97·9

Dose and Group	*Number of Pigs*	*Weight in lb. at beginning of experiment.*	1	2	3	4	5	6	7	8
Group I	1281	27	27	28	28	29	30	34	38	3
12 weeks	1269	50	48	51	56	63	70	81	85	9
0·5 cc.	1282	28	28	26	28	29	32	36	40	4
$1·69\times10^6$	1273	38	37	40	40	44	48	57	60	6
	1277	28	30	32	34	36	41	49	55	6
Group II	1283	28	32	31	34	38	37	42	43	4
12 weeks.	1271	44	48	51	55	60	66	73	76	8
0·05 cc.	1284	32	33	36	40	43	48	65	64	6
$16·9\times10^6$	1272	35	42	45	48	53	58	67	71	7
	1279	32	36	38	40	45	49	54	60	5
Group III	1292	47	52	52	57	67	74	88	97	10
8 weeks.	1293	20	23	20	20	21	21	25	28	3
0·05 cc.	1295	41	45	49	55	63	69	71	75	8
$16·9\times10^6$	1302	36	38	36	41	46	50	58	56	6
	1301	30	33	32	35	39	46	51	53	6
Group IV	1280	29	33	36	39	45	51	58	59	6
12 weeks.	1274	41	45	48	51	57	61	71	79	8
0·005 cc.	1287	29	34	36	39	44	52	60	63	6
$1·69\times10^6$	1270	41	46	51	54	61	65	69	78	8
	1276	32	34	40	45	49	54	63	68	7
Group V	1288	40	42	46	51	55	63	69	76	8
8 weeks.	1290	40	43	48	51	55	62	71	71	7
0·005 cc.	1297	30	31	36	37	40	46	51	53	6
$1·69\times10^6$	1298	32	40	43	48	52	59	63	73	7
	1296	31	32	34	38	42	47	52	52	6
Group VI	1285	30	31	36	40	42	47	50	56	6
12 weeks.	1268	46	50	59	66	72	78	85	96	10
Controls	1286	30	32	39	44	48	58	63	69	7
No	1275	32	37	43	49	52	54	62	71	7
infection	1278	32	38	42	46	50	57	64	68	7
Group VII	1289	45	49	56	59	66	73	81	89	9
8 weeks.	1291	46	52	61	65	74	82	92	98	10
Controls	1299	29	31	36	39	42	48	52	56	6
No	1300	27	30	35	38	41	42	48	51	5
infection	1249	27	31	34	37	41	44	50	53	5

Experiment 3

Two litters totalling 18 pigs were used. One litter was 53 days and the other 65 days old at the beginning of the experiment. Owing to illness of the sow the pigs, which were 53 days old, had to be weaned at 37 days but did well. The pigs were divided into three groups and housed in adjacent brick sties (see Experiment 2). As in the other experiments, the symptoms of infection appeared after the same interval. The results are given in Table III.

Experiment 4

The object of this experiment was to compare the effect of the same intensity of infection on pigs of different ages. Four litters were used, totalling 35 pigs, divided into six groups. The older pigs were 84 and 85 days old and the younger 60 and 62 days. They were kept in brick sties as in the last experiment. Unfortunately the younger pigs were of better quality than the older and grew more rapidly as will be seen from the average weights. The results are shown in Table IV.

Experiment 5

This experiment was undertaken to compare the effects of very small amounts of culture (0·0005 cc.) in single and repeated doses. Three litters, totalling 26 pigs, were divided into three groups of eight, two animals being rejected. The infecting dose was approximately 200,000 *S. suipestifer* organisms given once to one group and repeated daily for ten days for the other group, the total number of organisms being the same as in the lowest dose (0·005 cc.) in previous experiments. The third group was the uninfected control. The infecting dose was given with a syringe by the mouth. The other groups were given water with the syringe to allow for possible weight disturbance caused by the repeated handling of the pigs. As before the pigs were kept in adjacent brick sties.

Results. Diarrhoea was first seen after four days in the group which had received repeated doses. At this time the single dose group was less anxious than normal for food but had no diarrhoea. The following day several of the pigs in the repeated dose group still had diarrhoea and were feeding badly and one pig in the single dose group had diarrhoea. By the sixth day the single dose group was feeding well though one pig still had diarrhoea. The repeated dose group were coming back on feed although they were still being infected. By the eleventh day, *i.e.* the day following the last infection in the repeated dose group, all the pigs were feeding well and diarrhoea had ceased. The results are shown in Table V. The weighing was discontinued at six weeks because the pigs had obviously returned to normal.

Table V.

Average Weight Gains of Infected Groups Expressed as a Percentage of Weight Gains of Uninfected Controls.

Group	*Dose*	*Average weight in lb.*	*Weeks after infection*					
			1	*2*	*3*	*4*	*5*	*6*
Repeated dose	0·0005 cc. 191 × 10^4 10 times	41·9	51·61	79·48	83·33	86·13	99·37	100·00
Single dose	191 × 10^4 0·0005 cc.	42·1	90·32	77·55	89·88	92·85	101·25	102·36

Dose and Group	*Number of pigs*	*Weight in lb. at beginning of experiment*						
			1	*2*	*3*	*4*	*5*	*6*
Group I 10 doses 0·0005 cc. 191 × 10^4 (Total)	2426	61	64	68	79	87	98	112
	2423	31	34	40	44	49	58	68
	2436	46	50	50	61	68	82	92
	2432	44	45	51	58	64	73	83
	2438	37	35	37	40	48	62	72
	2439	44	49	57	65	72	82	92
	2446	42	40	45	56	62	75	87
	2444	30	31	37	44	49	60	68
Group II Single dose 0·0005 cc. 191 × 10^4	2425	53	54	62	70	74	86	97
	2429	33	38	41	46	54	65	78
	2430	53	57	60	71	80	89	101
	2434	42	44	49	58	65	70	82
	2435	39	40	44	52	60	72	83
	2443	44	47	55	62	70	81	94
	2448	38	42	47	53	59	72	82
	2445	35	37	40	46	52	61	66
Group III Controls No infection	2428	42	47	55	61	67	74	88
	2427	40	43	49	53	59	68	77
	2431	57	62	68	76	86	98	110
	2433	41	41	49	57	62	68	77
	2437	40	41	46	52	59	67	75
	2440	42	46	53	61	69	77	87
	2442	42	44	52	61	66	74	82
	2441	31	36	42	49	58	65	78

Weighings discontinued at sixth week because pigs had obviously recovered completely.

DISCUSSION

From an examination of the data in the tables there can be no doubt that a suitable dose of culture infected the pigs and that the size of the dose was generally reflected in the severity of the infection as shown by the loss of weight. The 0·5 cc. dose produced a disease similar to that seen in the field whilst the comparatively minute dose used in Experiment 5, although transitory in effect, definitely infected the pigs. It cannot be claimed that a standard dose was found because approximately similar numbers of organisms varied in effect. It is impossible to assess how much of this variation was due to the experimental conditions and how much due to real resistance or susceptibility. In none of the experiments described was the disease as severe as that produced by spraying the grass. It will be obvious that if a control group is skilfully fed or is of such good quality as to thrive well under adverse conditions, the apparent effect of the infection on the other groups will be magnified. Conversely, it will be diminished by poor pigs or by unskilful feeding. Genetic factors may also have played a part. In pigs it is impossible or difficult to get a constant genetic composition such as Webster obtained in his mouse experiments. What is clearly shown is that relatively small doses of culture did produce an effect. In any group the severity of the damage to the bowel varied widely as shown by post-mortem examination of those that died; moreover, there was a wide divergence in the weight loss of different pigs. Similar difference occur in most field outbreaks in which the number of deaths is small. The death of a few pigs is, however, of minor importance when compared with the failure to gain weight after infection. In most of these experiments the effect was prolonged and was still reflected in the last weighing. The serious effects of this disease on the success of pig rearing needs no stressing. Few pig farmers keep accurate records of the efficiency of food conversion and realise the loss caused by infections so mild as to be hardly apparent clinically.

The number of organisms in the same volume of the infecting doses varied in different experiments. This factor was not, however, of great importance since the maximum variation was only about two to one, whereas the difference between the infecting doses was ten to one. In Experiment 1 the pigs were at grass and they had to contend with secondary infection from faeces. In the other experiments the pigs were kept in brick sties which were cleaned daily.

It was somewhat astonishing to find that a dose of 0·0005 cc. of culture was sufficient to infect the pigs. Although only one pig had diarrhoea there was a general loss of weight. It was expected that the repetition of this dose daily for ten days would cause severe infection but, in fact, the effect was little more marked than the single dose, although nearly all the pigs in this group had diarrhoea. It is impossible to estimate the number of organisms

which will set up the disease in pigs under natural conditions, but if the experimental results are any guide, the number must be large. It is apparently not the repetition of the infection but the size of the individual dose which is important. Clinically, the pigs which received ten doses of 0·0005 cc. were recovering while still being infected. In the field pigs must continually pick up infection over a period, become infected and recover.

In Experiments 1 and 3 a dose of 0·005 cc. had a serious effect on the pigs. In Experiment 4 this dose had relatively little effect on the eight-and-a-half-weeks-old pigs and still less on the twelve-weeks-old pigs. It may be noted, however, that the younger and older groups of pigs in Experiment 4 had the same average weight per group although there was a difference of four weeks between them. The disease caused by this dose was rarely so severe as in natural outbreaks, but there was one death in Experiment 1.

In Experiment 4 a dose of 0·05 cc. had a mild effect on eight-and-a-half and twelve-weeks-old pigs. Similar results were obtained in the twelve-weeks-old pigs in Experiment 2. In Experiment 1 and 3, in which eight-and-a-half and nine-and-a-half weeks old pigs were used, the effect was severe and prolonged, with one death in each group.

A dose of 0·5 cc. can be relied on to cause severe disease, especially in the younger pigs. In Experiment 1 there were three deaths in the nine-and-a-half-weeks-old pigs. Although it is true that the smaller dose of 0·05 cc. caused a similar loss in the weight, there was no doubt about the effectiveness of a dose of 0.5 cc. in the two experiments in which it was used on twelve-weeks-old pigs.

As already stated, there was considerable variation between individual pigs and the original intention to use ten pigs per group was to submerge the individual variation by the group effect. The rate of recovery also varied widely. It was quite easy at every stage to see the differences between the groups. It was obvious that some pigs had suffered permanent injury and would never return to normal, while others recovered rapidly and subsequently gained weight at the same rate as uninfected pigs.

The post-mortem and bacteriological findings were similar to those in animals from natural outbreaks. The acute septicaemic form with rapid death did not occur in any of the animals given the graded doses, but one animal exposed to grass infection died from this type of infection. This septicaemic form is rare in field outbreaks, but is mentioned here because the lesions are identical with those of acute swine fever.

CONCLUSIONS

Preliminary experiments showed that pigs at grass which were not suffering from any known nutritional defect could be infected with *S. suipestifer*. The main experiments were designed to find a dose which would produce a disease similar to that which occurs

in the field, and which could be used as a basis for nutritional and chemotherapeutic experiments.

Groups of five or ten pigs, eight to twelve weeks old, *i.e.* at the susceptible age for necrotic enteritis, were infected with approximately 200,000 to 400,000,000 *S. suipestifer* by mouth. They were observed over a period of eight weeks following infection, and the effect was measured by difference in weight gain as compared with uninfected controls. The mortality was low, but the disease was similar to that seen in the field.

It was found that pigs could be regularly infected with a comparatively small number of *S. suipestifer*. The effect of the infection was clearly shown by the degree of failure to gain weight. The amount of weight lost was, in general, proportional to the size of the infecting dose, but was influenced by other factors such as constitution, age and weight at weaning.

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THE EFFECT OF ADRENAL CORTICAL EXTRACTS ON THE IMMUNITY RESPONSES OF THE RABBIT

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INTRODUCTION

The effects of the adrenal steroids upon the immunological responses of animals are as yet little understood and the literature contains conflicting reports.

Fox and Whitehead (1936) claimed that there was an increased antibody content of serum when animals were simultaneously injected with antigen and adrenal cortical extracts. Dougherty and White (1945) observed an involution of lymphoid tissue in animals treated with adrenocortical extracts and they claimed that this was accompanied by a release of antibodies from the lymphoid tissue. Dougherty, Chase and White (1945) described an anamnestic response in rabbits following a single injection of adrenal cortical extracts. Chase, White, and Dougherty (1946) asserted that in rabbits, rats, and mice, the rate of antibody production was increased when adrenocortical hormones were given simultaneously with antigen, and that in hyperimmunised rabbits a single injection of adrenocortical hormone would double the antibody content of the serum. They stated that these changes were attributable to the breakdown of the lymphoid tissue and the consequent release of antibody. Clarke and Jacobs (1950) noted a very slight increase in antibody content in rabbits given aqueous adrenal cortical extracts, but said that injections of lipoidal extracts had no effect.

On the other hand, Eissen, Mayor, Moore, Tarr, and Stoerk (1947), reported that adrenocortical extracts had no effect upon serum antibody content or upon any other fraction of the serum protein, and concluded that "adrenal cortical activity is not essential for the fabrication or release of antibodies or gamma globulins". These findings were confirmed by Thatcher, Houghton, and Zeigler (1948). Indirect confirmation was given by Philips, Hopkins, and Freeman (1947), who noted that there was no increase in antibody content when lymphoid tissue was destroyed by nitrogen mustards. Murphy and Sturm (1947) adrenalectomised rabbits and produced lymphoid hyperplasia, and in these cases there was increased antibody production. Volmer and Samsell (1949) injected mice with pneumococcal antigens and adrenocortical hormones and then tested the resultant immunity. They concluded that the adrenocortical extracts had no effect upon the resistance of the mice. Saunders and Adams (1950) treated patients with cortisone and adrenocorticotrophic hormones and recorded no changes in the antibody content of the patients' sera.

Bjørnboe, Fischel, and Stoerk (1951) found that the administration of adrenocorticotrophic hormone and of cortisone caused a reduction in the concentration of antipneumococcal antibody in the circulation of rabbits. These workers noted this effect of adrenocorticotrophic hormone (ACTH) and adrenocortical hormone (ACH) both during immunisation and after immunisation was well advanced.

The work presented here was undertaken in order to determine what effects, if any, might be produced by ACH on the antibody responses of rabbits. Two antigens were used together with two preparations of ACH. Nitrogen mustard hydrochloride was used as an example of a substance which would destroy lymphoid tissue and which might therefore help in the interpretation of the action of ACH.

METHODS

Preparation of the antigens

Salmonella pullorum. The strain used was one recently isolated from chicks. A suspension of the organism in normal saline to a final density of 2044^6 organisms per ml. was heat treated at 80° C. for one hour on three consecutive days. It was felt that this severe heat treatment might result in the liberation of endotoxin from the bacterial cells and thus increase the antigenic activity of the suspension.

Brucella abortus. The standard suspension as issued by the Ministry of Agriculture and Fisheries, Weybridge, was diluted 1/10 at the time of use.

Agglutination tests

The agglutination tests for *S. pullorum* and *Br. abortus* antibodies were done in the usual manner. The tubes were incubated over-night at 37° C. and allowed to stand for 30 mins. at bench temperature before reading. The end point in each case was taken as the last tube with a completely clear supernatent fluid.

White Cell Counts

These were performed in the usual manner using a haemocytometer with Neubauer reading.

Experimental Animals

Twenty adult rabbits comprising six females and fourteen males were paired off according to sex and weight. The rabbits were bled prior to the commencement of the work and normal white cell counts established. All were examined for the presence of antibodies against *S. pullorum* and *Br. abortus* with negative results. The process of immunisation was carried out by subcutaneous injections of the antigens. ACH preparations were given by intramuscular injections.

Adrenal cortical hormones

The following preparations were used:

"Eschatin". This was an aqueous preparation "each cc. of which contains the equivalent biological activity of 200 mg. of 11-dihydro-17 hydroxycorticosterone (Compound E. of Kendall)."

"Lipoadrenal" (Upjohn). This was an oily extract which contained "Corticosterone, 17-hydroxycorticosterone, 11-dihydrocorticosterone and 11-dihydro- 17-hydroxycorticosterone."

EXPERIMENTAL OBSERVATIONS

The effects of ACH upon the antibody response to S. pullorum

The effects of "Eschatin" during immunisation

In healthy rabbits the injection of Eschatin produces a slight leucopenia and some temporary damage to lymphoid tissue. Earlier workers had claimed that the simultaneous injection of ACH and antigen resulted in an increased rate of antibody production and also an increased antibody content of serum.

Ten pairs of rabbits were used. One group received *S. pullorum* antigen alone and the other group was given *S. pullorum* and "Eschatin" simultaneously. The white cell counts and the antibody titres were followed during the experiment. The changes in the leucocyte counts in the two groups may be seen in Table I.

TABLE I

EFFECT OF A.C.H. ON THE ANTIBODY RESPONSE TO *S. pullorum*

Date.	*White Cell Count in thousands per cmm.*	
	S. pullorum	*S. pullorum plus "Eschatin"*
14/4/50	8·6	8·7
17/4/50	8·2	6·9
18/4/50	11·4	8·8
19/4/50	14·9	10·7
21/4/50	21·0	13·8
24/4/50	11·6	8·5
26/4/50	11·0	8·1

The leucocyte counts represent the mean figures for each group of rabbits. They show that the ACH group had a reduced leucocytic response. Individual leucocytic responses varied a good deal, but in all cases ACH prevented the development of a leucocytosis.

The changes in the antibody titres may be seen in Fig. 1. In seven pairs the animals which received antigen alone developed higher titres than those which had antigen and Eschatin, and in the other three pairs the titres of both animals were the same. There were no appreciable differences in the rate of antibody production (as judged by the presence of circulating antibody), or in the rate at which antibody disappeared from the circulation.

The effect after immunisation

Dougherty *et al* (1945) reported that the inoculation of ACH into previously immunised rabbits gave rise to an anamnestic

FIG. 1.

The effect of "Eschatin" on the antibody response to S. pullorum

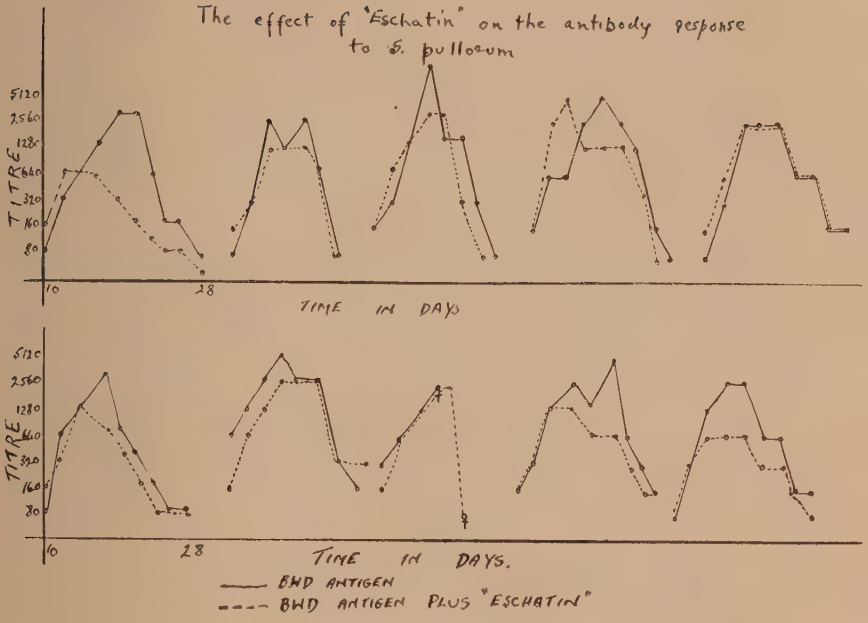
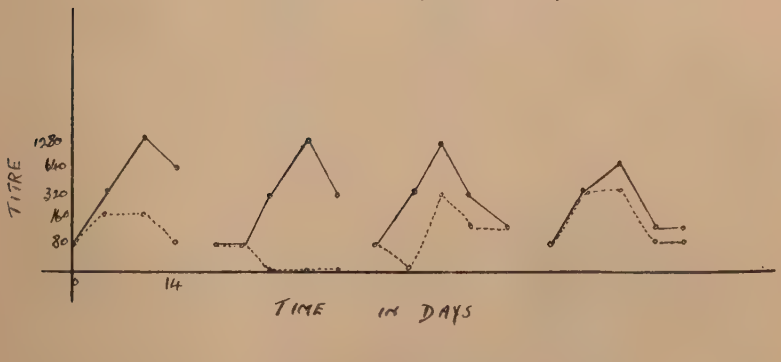


FIG. 2

The effect of "Eschatin" on rabbits previously immunised against S. pullorum



response. It was therefore proposed to try the effect of Eschatin and Lipoadrenal on rabbits previously immunised against *S. pullorum*.

Four pairs of the rabbits used in the preceding experiment were selected, each of which had an antibody titre of not more than 1/80 against *S. pullorum*. The four rabbits which had previously received antigen plus Eschatin were now given a single injection of antigen and the remaining four were given a single inoculation of Eschatin. The antibody titres were again followed.

The results (Fig. 2) show the marked response to antigen and the negligible result in the Eschatin group.

The Effect of Lipoadrenal on rabbits immunised against S. pullorum

Sixteen rabbits which had previously been immunised against *S. pullorum* were paired off according to sex and weight. Nine were given a single inoculation of 1·0 ml. Lipoadrenal and seven an inoculation of 1·0 ml. of a suspension of equine red cells. The antibody titres for salmonella agglutinins were determined immediately before injection and at six and twenty-four hours after inoculation. The results are shown in Table II.

TABLE II

EFFECT OF LIPOADRENAL ON ANTIBODY RESPONSE TO *S. pullorum*

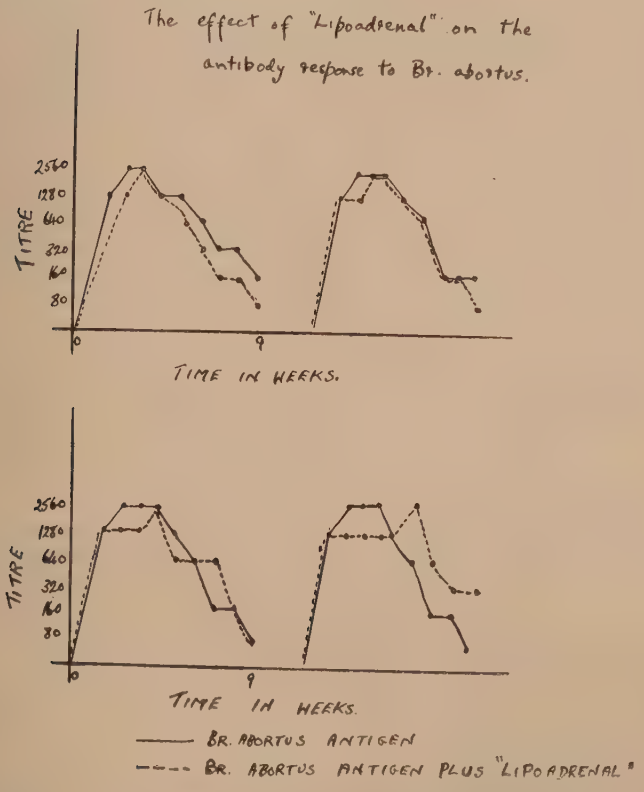
Inoculum	*Initial Titre*	*Titre after 6 hrs.*	*Titre after 24 hrs.*
" Lipoadrenal "	1/40	1/40	1/80
"	1/10	1/10	1/80
"	1/20	1/10	1/320
"	1/20	1/20	1/80
"	1/20	1/20	1/40
"	1/320	1/640	1/640
"	1/320	1/640	1/640
"	1/80	1/80	1/160
"	1/40	1/40	1/40
" Equine Red Cells "	1/10	1/10	1/10
"	1/20	1/20	1/20
"	1/20	1/20	1/80
"	1/20	1/20	1/40
"	1/10	1/10	1/10
"	1/40	1/40	1/40
"	1/20	1/20	1/10

The injection of equine red cells increased the antibody response in two rabbits only whereas eight of the nine rabbits given Lipoadrenal extract showed a definite rise in titre. This suggested that the increased antibody content was due to the specific effect of the A.C.H. and was not merely due to the injection of foreign protein.

The effect of "Lipoadrenal" on the antibody response to Brucella abortus

Eight rabbits with sera negative for *Br. abortus* agglutinins were paired off and one of each pair received a series of inoculations of *Br. abortus* antigen; the remaining four were inoculated with the same antigen plus 1·0 ml. Lipoadrenal. The antibody titres of these rabbits were then followed for a period of nine weeks. The results are shown in Fig. 3.

FIG. 3



It will be seen that the simultaneous injection of ACH and antigen had no appreciable effect upon the antibody content of the sera of these rabbits.

The effects of Nitrogen Mustard Hydrochloride on circulating S. pullorum *antibody*

Nitrogen mustard hydrochloride damages lymphoid tissue and

therefore in this respect resembles the action of ACH. Philips *et al* (1947) claimed that the inoculation of nitrogen mustards resulted in a decreased serum antibody content.

Eight rabbits were used in this experiment. These rabbits had previously been immunised with *S. pullorum* and came from the control group in the experiment with Eschatin. The titres for Salmonella agglutinins were determined immediately before an intravenous injection of 0·2 mg. nitrogen mustard hydrochloride and again, twenty-four hours later. The results are shown in Table III.

TABLE III

EFFECT OF NITROGEN MUSTARD ON ANTIBODY RESPONSE TO *S. pullorum*

Inoculum	*Initial Titre*	*Titre after 24 hrs.*
0·2 mg. Nitrogen Mustard Hydro-chloride	1/160	1/80
„	1/10	1/10
„	1/10	1/40
„	1/40	1/40
„	1/40	1/40
„	1/80	1/40
„	1/160	1/160
„	1/80	1/80

The results indicate that the intravenous injection of nitrogen mustard hydrochloride had no demonstrable effect upon the antibody content of the serum.

DISCUSSION

The results obtained in these experiments indicate that in so far as the two antigens selected are concerned, adrenal cortical extracts do not increase the antibody content of the sera of rabbits to any significant extent.

These negative findings do not agree with the results obtained by Fox and Whitehead (1936) and Chase *et al* (1946), but support those of Volmer and Samsell (1949) and Bjornboe *et al* (1951). Dougherty (1945) described an anamnestic response in rabbits after the injection of adrenal cortical extracts. In the case of Eschatin and *S. pullorum* antibody this anamnestic response was not noted, but with " Lipoadrenal " there was a slight but definite increase in titre. This is of interest, since Clarke and Jacobs (1950) stated

that aqueous extracts were capable of giving slight increases in antibody content whereas lipoidal extracts were not. In these experiments exactly reverse results were noted.

It has been suggested that the cortical extracts act by causing dissolution of the lymphoid tissue and the lymphocytes and thereby releasing antibody (Chase *et al,* 1946). If this were the case then other agents which damage lymphoid tissue might be expected to produce the same results. Nitrogen mustard hydrochloride has a powerful destructive action on lymphoid tissue, but in the experiments described in this paper this substance failed to show any significant effect upon serum antibody levels. Similar results were obtained by Philips, Hopkins and Freeman (1947).

The general impressions gained from these experiments was that adrenal cortical extracts had either no effect upon antibody titres or were responsible for a slight inhibition. This inhibition is not perhaps surprising, since adrenal cortical extracts damage lymphoid tissue and such damage may interfere with its functions.

SUMMARY

Two preparations of adrenocortical hormone had no appreciable effect upon the antibody responses of rabbits to *S. pullorum* and *Br. abortus* antigens respectively.

Nitrogen mustard hydrochloride had no effect upon circulating *S. pullorum* antibody.

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CHRONIC CANINE NEPHRITIS

II. A Study of the Parathyroid Glands with Particular Reference to the "Rubber Jaw" Syndrome*

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INTRODUCTION

Hyperplasia of the parathyroid glands is a well-known accompaniment of chronic renal impairment in the human subject and has been carefully studied by Pappenheimer and Wilens (1935), Gilmour and Martin (1937) and Gilmour (1947). It has been shown experimentally that acute renal failure in rats produced by bilateral nephrectomy, causes a pronounced increase in the mitotic activity of the parathyroid glands (Platt, 1950). Renal impairment of longer duration, produced by partial destruction of kidney substance in rats, is associated with enlargement of the glands (Pappenheimer, 1936). Liegeois and Derivaux (1946) have described an increase in the weight of the parathyroid glands of dogs suffering from chronic nephritis.

The present paper is devoted to a study of the position, number, size and weight of the parathyroid glands in dogs which are free from renal or bone disease and also the weights of these glands in dogs suffering from chronic nephritis with and without the "Rubber Jaw" syndrome.

Throughout this paper, the term "chronic nephritis" is employed to connote a condition in which the kidneys may show extensive scarring and replacement fibrosis, together with tubular atrophy and distortion. The more general term "renal fibrosis" would be more strictly appropriate to this type of lesion. The condition is quite consistent with the late sequelae of an acute leptospiral nephritis in which extensive destruction of the renal parenchyma has been followed by replacement fibrosis and scarring. Nevertheless, similar changes might be produced by several different types of antecedent condition, and in those kidneys which show extensive metastatic calcification it is particularly difficult to exclude the possibility that the calcium deposits had played a part in provoking the development of fibrous tissue. Renal fibrosis, excited by metastatic calcification, calculus formation and sometimes infection, is a frequent complication of hyperparathyroidism in man, engendered by adenomata and primary hyperplasia of the parathyroid glands (Albright, Baird, Cope and Bloomberg, 1934).

Metastatic calcification was present in the kidneys of two animals in the Rubber Jaw group and two of the chronic nephritis

* This paper formed a part of a thesis presented to the University of Liverpool for the degree of M.V.Sc.

group but the changes in the parathyroid glands and bones in these dogs were indistinguishable from those where metastatic calcification was minimal or absent. Although the possibility cannot be entirely excluded that metastatic renal calcification may occur, and be followed by resorption of the calcium salts at some subsequent date, it seems likely, on the whole, that in most or all of the cases which are described in this paper, the renal scarring and the fibrosis, with consequent excretory impairment, were the primary factors in the pathogenesis of the parathyroid hyperplasia.

NATURE OF MATERIAL

The non-nephritic group comprised 18 dogs in none of which was there any clinical evidence of bone disease. The ages of the dogs was not known but almost all were adults. In all these animals the kidneys were examined histologically and shown to be free from nephritis or post-nephritic scarring.

The chronic nephritic group consisted of five adults, all typical unselected examples of the condition. All these animals were free from clinical bone disease, but osteodystrophy of varying severity was apparent histologically. Extensive metastatic calcification was present in the kidneys of two dogs.

The "Rubber Jaw" group included seven adults, all with severe chronic nephritis complicated by softening of the jaw bones. A severe degree of metastatic calcification of the kidneys was also present in two of these animals.

Descriptions have been given elsewhere of the histological changes in the kidneys in canine chronic nephritis (Platt, 1951a). The histopathology of the bone lesions is also the subject of a separate paper in which the relationship of Rubber Jaw to chronic nephritis is discussed (Platt, 1951b). It will there be pointed out that osteodystrophy is a frequent though unsuspected accompaniment of many cases of chronic nephritis and that the Rubber Jaw syndrome only arises when these bone changes are unusually severe.

All the animals with nephritis (groups 2 and 3) showed clear evidence of renal insufficiency at the time of death or destruction judged by the blood urea level or the clinical picture.

METHODS

In all the dogs, no attempt was made to isolate the parathyroids while they were still in the fresh state at autopsy. Instead, the trachea and the thyroid glands, with the attached parathyroids, were removed in one piece and fixed in 10 per cent. formol-saline solution for three to four weeks. The parathyroid glands were then dissected and their positions noted; in many cases, their diameters were measured. After separation, they were dried between pieces of absorbent paper and immediately weighed on a microbalance.

RESULTS

The Parathyroid Glands in Normal Dogs

A careful examination of the parathyroid glands of normal dogs is essential before any opinion can be formed as to whether these glands have undergone any sort of change in dogs with chronic nephritis. This is all the more important since the parathyroids of the dog are small, often difficult to find and are very variable in size, number and position.

Position of the parathyroid glands in normal dogs.

There is a considerable variation, both in the number and position of the parathyroid glands of the dog. Godwin (1937a) has described the origins and usual variations that occur in this animal. Briefly, the parathyroid glands on each side are derived from the dorsal part of the third and fourth branchial pouches. The third pouch gives origin to the anterior or external gland, while the fourth pouch is the source of the posterior or internal gland. These will be referred to as parathyroid III and parathyroid IV respectively. According to Godwin, parathyroid III is usually found on the lateral surface of the thyroid gland at the level of the anterior third of that gland. Not infrequently it is found at the anterior extremity but it may be located at any level, even at times near the extreme posterior pole. It usually rests in a slight depression in the thyroid gland. Accessory parathyroid glands are often present on the surface of the thyroid and are usually large and quite close to the parent gland. The main parathyroid IV on the other hand is located on the medial surface of the thyroid in the anterior part of the posterior half of the gland and it tends to be near the dorsal border. In a few dogs it may be found on the lateral surface near the dorsal border. Its location is, however, subject to considerable variation. Godwin (1937b) states that up to 14 accessory parathyroids IV may be present but this observation was based upon microscopical examination of serial sections of the whole thyroid lobe, a method which will reveal numerous accessory glandules embedded in the thyroid gland which would not be visible on its exterior even if they were of macroscopical size. However accessory parathyroids IV of macroscopical dimensions do occur not infrequently.

In the present investigation, the thyroids and the structures in their neighbourhood, after preliminary fixation, were dissected with considerable care. Only tissue which was unequivocally of parathyroid origin was taken into account. As a rule, the glands could readily be recognised by their firmness, their rather smooth shiny surface and a tendency to shell out from the thyroid in whose surface they were often partly embedded. They were usually of a creamy colour but in a small proportion of cases they were a deep brown.

It is of some interest to note that in six out of eighteen animals,

careful search at the anterior pole of both the left and the right thyroid glands failed to reveal any sign of parathyroid tissue. In another animal there was only a very minute glandule at the anterior pole. In most of these cases, the absence or small size of the anterior glands was compensated for by large accessory parathyroids III, up to three in number. In one instance, a normal sized parathyroid III was present on one side only. Another animal showed division of the parathyroids III on each side into multiple small glandules, six on the right side and four on the left. All these were situated in groups at the anterior poles of the thyroids. In one case, no evidence of parathyroid IV tissue could be found.

The total number of discrete glands found in these animals, taking both left and right sides into consideration is shown in Table I, from which it is clear that the modal number of glandules

Table I

Frequency of Occurrence of Multiple Parathyroid Glandules in Normal Dogs.

Number of glandules present	*Frequency of occurrence*
3	2
4	6
5	3
6	5
7	1
13	1

is four; however these need not necessarily consist of one parathyroid III and one parathyroid IV, on each side, and in the present series this arrangement was found to occur only occasionally.

Sizes of the normal parathyroid glands.

In conformity with the variation in the number of parathyroid glandules which may be present, there is a similar wide diversity in their size and in the animals which have been examined they were found to range from minute bodies 0·5 mm. or less in diameter up to maxima of $4 \times 2{\cdot}5 \times 2$ mm. (parathyroid III) and $4 \times 3 \times 2$ mm. (parathyroid IV). The mean values were as follows:

Parathyroid III : $2{\cdot}4(\pm{\cdot}8) \times 1{\cdot}8(\pm{\cdot}6) \times {\cdot}9(\pm{\cdot}5)$ mm.
60 glandules were measured.
Parathyroid IV : $1{\cdot}8(\pm{\cdot}6) \times 1{\cdot}3(\pm{\cdot}5) \times {\cdot}6(\pm{\cdot}4)$ mm.
29 glandules were measured.

(The dimensions of the parathyroid glandules in one animal (No.

14) exceeded the above maximal values, but for reasons stated in the next paragraph, this observation has been excluded.)

In shape, the glandules were either spherical or ovoid, or flat leaf-like ellipsoids 0·5 mm. or less in thickness. In view of the variety of different shapes, linear measurements are not a suitable means of assessing the amount of parathyroid tissue that is present.

The weights of the normal parathyroid glands.

The weights of the parathyroid glands in 13 male and 5 female dogs were determined as described. When the parathyroid weights in milligrams were plotted against the respective body weights in kilogrammes, all the points with one exception fell within the limits 0·44 to 2·31 mg. per kilo. The single discordant observation was derived from a female Bulldog, and here the weight of the parathyroid glands was greatly in excess of what might be expected in a dog of that body weight, judged by the values for the remainder of the group. This observation has therefore been excluded from any calculations since on statistical grounds there is a high degree of probability that the parathyroid glands in this animal were either abnormal or unrepresentative of the group. It is possible that the parathyroid glands in Bulldogs are proportionately larger in relation to body weight than in other breeds whose skeletal development approaches more closely to the modal type. The values obtained for each dog (including the discrepant observation—number 14) are shown in Table II.

Table II

Relation Between Parathyroid Weight and body Weight in 18 Dogs free from Renal Disease.

No.	*Breed*	*Sex*	*B.Wt. (Kg.)*	*Parathyroid wt. (mg.)*	*Mg. of parathy tissue/Kg.B.W.*
1	Mongrel	F	10·21	14.0	1·37
2	Spaniel	M	15·65	21·0	1·34
3	Mongrel	M	6·80	3·0	·44
4	Smooth haired terrier...	M	16·33	25·0	1·53
5	Smooth haired terrier...	M	11·11	22·5	2·02
6	Smooth haired terrier...	M	15·42	26·0	1·68
7	Cairn terrier	M	9·75	22·5	2·31
8	Irish terrier	M	13·61	21·5	1·57
9	Mongrel	F	9·98	10·5	1·05
10	Mongrel	F	10·21	4·5	·44
11	Mongrel	M	7·71	5·5	·71
12	Chow	M	12·93	11·5	·88
13	Mongrel	M	11·79	10·5	·89
14	Bulldog	F	15·88	72·5	4·56
15	Smooth haired terrier...	M	11·57	13·5	1·17
16	Mongrel	M	7·03	9·5	1·35
17	Spaniel	M	7·03	15·5	2·20
18	Mongrel	F	9·75	21·5	2·20

There was no significant difference in the weight of parathyroid tissue per kilogramme between male and female dogs.

The coefficient of correlation between total parathyroid weight and body weight was calculated and was found to have the significant value of $+0{\cdot}674 \pm 0{\cdot}250$. Comparisons of parathyroid size therefore should always be expressed in terms of the weight of gland tissue per kilogramme of body weight. The mean weight of the parathyroid tissue in the present series was $1{\cdot}36 \pm 0{\cdot}57$ mg./Kg. (When the discordant observation is also included the value becomes $1{\cdot}54 \pm 0{\cdot}92$ mg./Kg.)

The Parathyroid Glands in Chronic Renal Disease and Rubber Jaw

In all cases of renal disease which had persisted for any considerable length of time, and particularly in those cases accompanied by Rubber Jaw, there was clearly observable enlargement of the parathyroid glands. This could be seen in many cases on direct inspection and was confirmed by weighing the glands. In some cases of Rubber Jaw, the glands were not weighed but their enlargement was immediately apparent at the time of the gross post-mortem examination, to such a degree that no hesitation was felt in placing them well outside the normal range of size. In all the other instances the glands were weighed and measured.

The sizes of the parathyroid glands in chronic nephritis and in Rubber Jaw

The dimensions of the parathyroid glands in these two conditions are shown in Table III.

TABLE III

SIZES OF THE PARATHYROID GLANDS IN CHRONIC NEPHRITIS AND IN RUBBER JAW (in millimetres).

Group		*Parathyroid III*	*Parathyroid IV*
Chronic nephritis	Maximal size:	6 × 5 × 4	4 × 2·5 × 2·5
	Mean size:	4·2 (±1·5) × 3·3 (±1·2) × 2·1 (±1·1) 12 glandules measured	2·7 (±·9) × 2·1 (±·6) × 1·5 (±·6) 10 glandules measured
Rubber Jaw ...	Maximal size:	8·5 × 6·5 × 3·5	6 × 4·5 × 3·5
	Mean size:	5·7 (±2·1) × 4·3 (±1·3) × 2·9 (±1·0) 12 glandules measured	4·4 (±1·1) × 3·4 (±·6) × 2·1 (±·7) 10 glandules measured

The weights of the parathyroid glands in chronic nephritis and in Rubber Jaw

The weights of the parathyroid glands and their relation to the body weight of the animal are shown in Table IV.

TABLE IV

PARATHYROID WEIGHT/BODY WEIGHT RATIOS IN RUBBER JAW AND CHRONIC NEPHRITIS.

No.	*Breed*	*Sex*	*B.W. (Kg.)*	*Parathyroid wt. (mg.)*	*Mg. parathyroid tissue/Kg. B.W.*
	A.—*Chronic Nephritis.*				
33	Bull terrier cross ...	M	14·7	146·5	9·9
34	Bull terrier	M	13·8	93·5	6·8
37	Sealyham	M	12·0	52·5	4·4
XI	Mongrel	M	15·0	44·5	2·9
38	Smooth haired terrier...	M	12·8	66·0	5·2
	B.—*Rubber Jaw.*				
29	Smooth haired terrier...	M	13·0	133·0	10·2
30	Mongrel	M	12·9	240·0	18·6
31	Smooth haired terrier...	M	14·9	142·5	9·6
32	Cross bred Chow ...	M	14·0	205·0	14·6

The coexistent chronic nephritis which was present in these cases was essentially similar in type, irrespective of whether the Rubber Jaw syndrome was also present or not. The kidney lesions did however tend to be more severe in those cases accompanied by clinical bone changes.

Since all the animals with chronic renal disease, either with or without Rubber Jaw in addition, weighed between 10 and 15 kg., the parathyroid weights of those animals in the above normal series falling within the same weight range, may be accepted for purposes of comparison (Table V).

TABLE V

COMPARISON OF PARATHYROID WEIGHTS IN NON-NEPHRITIC DOGS AND DOGS SUFFERING FROM CHRONIC NEPHRITIS AND RUBBER JAW OF THE SAME WEIGHT RANGE (10-15 KILOGRAMMES).

Group	*No. of animals*	*Mean weight of parathyroid tissue (mg.) per Kg. of body weight.*
Non-nephritic... ...	7	1·19 ± 0·48
Chronic nephritis ...	5	5·82 ± 2.39
Rubber Jaw	4	13·25 ± 3·64

Thus, compared with normal dogs of similar weight, the amount of parathyroid tissue per kilogramme body weight is very greatly increased in dogs suffering from chronic nephritis, and to an even greater degree in animals with Rubber Jaw. The series are small but the differences are outside the limits of probable chance occurrence.

The histological appearance of the parathyroid glands in Rubber Jaw and chronic nephritis

Material for histological examination was fixed in 10 per cent. neutral formol saline, dehydrated, embedded in paraffin and cut at a thickness of 6 micra. Sections were stained with haematoxylin and eosin. Frozen sections were also prepared and stained by Scharlach R.

No difference in histological appearance could be detected between the glands of animals with chronic nephritis without clinical bone changes and those complicated by Rubber Jaw. The main respect in which the glands of dogs with chronic nephritis and Rubber Jaw differed in any way from the normal was in the arrangement of the cells. In the dog, the cells of the parathyroid glands are normally disposed in the form of anastomosing strands and this arrangement persists throughout life. The present hyperplastic glands frequently showed this arrangement in parts of the gland. In many of the glands, however, much or sometimes almost all of the section of gland was composed of cells arranged in a much more compact fashion. In these areas, the cells tended to form dense masses although they still showed a tendency towards an alveolar arrangement. Cytologically, chief cells were the principal type present, but in both normal and hyperplastic glands, there were also numerous "small dark cells". These were often in groups, but they were also found scattered among the chief cells. They were smaller than the latter and had a rather irregular hyperchromatic nucleus. It is possible that they were chief cells at certain stages in their secretory cycle. Dark chief cells have been described by Marine (1932) in the parathyroid glands of normal dogs. No alteration was noted in the position of the chief cell nuclei, such as Castleman and Mallory (1935) observed in the hyperplastic glands of cases of von Recklinghausen's disease, where they found displacement of the nuclei towards the basal poles of the cells. Mitotic figures were scarce, but there was some nuclear hypertrophy. No intracellular fat was found in frozen preparations.

DISCUSSION

Chronic renal fibrosis is common among dogs and in many instances it is a sequel to acute infection of the kidneys by *Leptospira canicola* at some earlier date in the animal's history (McIntyre and Stuart, 1949).

The diffuse nature of the parathyroid hyperplasia which was present in these cases suggests that its etiology is related to the presence of some humoral stimulus affecting all parts of the glands equally. On the whole, the evidence suggests that the parathyroid hyperplasia is probably secondary to renal insufficiency arising from chronic nephritis and this appears to apply also in the case of Rubber Jaw, since this condition is distinguished from the majority of cases of chronic nephritis only by the greater extent of

the bone lesions and the parathyroid hyperplasia. At the same time, very little information is available regarding the possible occurrence of parathyroid adenomata in the dog, although the instance recorded by Perlmann (1944) may be an example of this condition. Detailed information on this point would be valuable.

The degree of parathyroid hyperplasia is probably related to the duration of time during which the provoking stimulus has been operative. In those cases where metastatic calcification of the kidneys is a prominent feature, it seems likely that this factor contributes to a further deterioration in renal efficiency and in consequence a further hyperplasia of the parathyroid glands.

SUMMARY

The position and number of parathyroid glandules has been studied in normal dogs.

The weight of parathyroid tissue per kilogramme body weight has been determined in normal dogs, dogs with chronic nephritis and dogs with the Rubber Jaw syndrome.

There is a significant increase in the weight of parathyroid tissue in chronic nephritis and a considerably greater increase in dogs with Rubber Jaw.

It was concluded that the parathyroid hyperplasia in these conditions was probably secondary to prolonged renal insufficiency.

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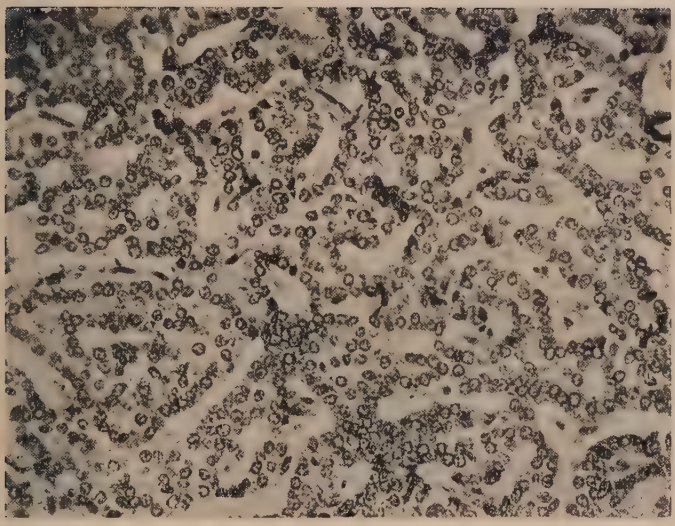

FIGURE 1.

Parathyroid gland of a normal dog. Note the loose trabecular arrangement of the cells and the numerous "small dark cells." H and E. × 400.

FIGURE 2.

Parathyroid gland of a dog with Rubber Jaw. The cells are more densely arranged than normally. There is some nuclear hypertrophy. H and E × 400.

CHRONIC CANINE NEPHRITIS

III. The Skeletal System in "Rubber Jaw"*

By

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INTRODUCTION

In the human subject, chronic renal insufficiency is not infrequently accompanied by widespread osteodystrophic changes in the skeleton (Ginzler and Jaffe, 1941). In the adult it is only rarely that the process is sufficiently severe to become clinically manifest. In children, however, renal impairment during the periods of active skeletal growth leads to the condition of "renal rickets", with stunting of growth and deformities in the long bones. Gilmour (1947) and Pappenheimer and Wilens (1935) have shown that chronic renal disease in man is usually accompanied by some degree of parathyroid enlargement.

In a small proportion of instances, severe chronic nephritis in the dog is accompanied by a very striking osteodystrophic softening of the jaw bones and the syndrome thus produced has been aptly designated "Rubber Jaw". The association of this condition with chronic nephritis was first commented upon by Hare (1934) and this observation has since been confirmed by other writers. The clinical aspects of this disease have been well described by Hogg (1948). In a previous paper (Platt, 1951b) it has been shown that canine chronic nephritis is usually accompanied by hyperplasia of the parathyroid glands and this is most pronounced in those dogs which are also affected with Rubber Jaw, in which the nephritis is complicated by palpable changes in the skeleton.

In the present investigation, a study was made of the gross and microscopical skeletal changes in seven adult dogs suffering from Rubber Jaw, and in seven other dogs affected with various stages of nephritis in none of which could clinical alterations in the skeleton be detected. With one exception in the latter group, all the dogs were males.

The histopathology of the renal lesions in these and other instances of canine nephritis have already been described (Platt, 1951a). The changes in the parathyroid glands in the present series of cases are also described elsewhere (Platt, 1951b).

METHODS

The methods used in the preparation of the bone sections consisted of fixation in 10 per cent. formol-saline solution for several weeks followed by treatment in 5 per cent. formic acid until decalcification was complete. After dehydration in alcohol,

* This paper formed a part of a thesis presented to the University of Liverpool for the degree of M.V.Sc.

the material was then embedded in paraffin and sections were cut 6 micra thick. They were stained as a routine with haematoxylin and eosin, and Masson's haematoxylin-Ponceau, fuchsin-light green method: occasionally the Azan modification of Mallory's method was also used. The skull of one animal was boiled in an attempt to display the alterations in the bones and this case was therefore not available for histological examination.

THE MORBID ANATOMY AND HISTOLOGY OF THE BONES IN RUBBER JAW

All the animals with Rubber Jaw showed the same type of osteodystrophic changes, but they varied from one dog to another in their intensity. The different parts of the skeleton were not affected with uniform severity; in all the dogs, much the greatest change was to be found in the bones of the skull where the disease process had advanced to such a degree that it was evident by gross examination. In none of the dogs was it possible to detect macroscopic changes in any other part of the skeleton; nevertheless, alterations similar to those in the skull, but less advanced, were detected histologically.

In the course of this study, attention has been directed principally to the changes in the skull and particularly to their site of appearance. Histological examinations have been made of other parts of the skeleton but only to an extent sufficient to establish the fact that the disease is a generalised process affecting all bony tissue; this fact would tend to be lost sight of if only changes which were apparent to clinical observation were taken into account.

The Changes in the Skull

Macroscopic Changes. On gross examination, the very great softening of the bones, particularly of the mandible and the superior maxilla, was at once obvious. The canine teeth could be moved to and fro without difficulty and on pressure the bone was soft and resilient. The extent of this palpable softening varied from one dog to another. In all, the jaws were affected, but sometimes the superior maxilla showed the change to a more pronounced extent than the mandible; in the rest of the skull, the softening sometimes extended to the nasal and frontal and other bones of the face, but here it was less obvious. In the very severely affected animals, however, such as No. 21, the whole of the skull could be felt to crepitate beneath moderate pressure of the hand; such severe change was not the rule. In two cases, Nos. 21 and 32, pronounced swelling of the head was a notable feature and the distortion thus produced in the normal architecture of the skull is well shown in Figure 1. From this it is clear that there was very serious encroachment on the cavity of the nasal chamber by the soft swollen superior maxilla. More frequently, however, the changes seen in the skull were those of thinning of the bone.

FIG. 1. Transection through the snout from a dog with Rubber Jaw. Note the gross thickening of the softened bone.

In the severely affected areas of either form the bone could be cut with ease by a scalpel. When an attempt was made to boil away the overlying soft tissues, it was practically impossible to achieve a clean separation as the periosteal fibrous attachments were intimately interwoven with the osteodystrophic fibrous tissue which formed the greater part of the underlying bone. The slightest traction on these fibrous attachments was enough to tear away the thin outer shell of bone cortex to which they adhered. It was noted that in the parietal bones the resorption was by no means a uniform process, but occurred to a greater extent in small pits up to a millimetre in diameter which were closely scattered throughout the whole plate of the bone, producing a speckled or worm-eaten effect when the bone was held against the light. Nowhere in the skull was there any sign of localised swellings, even in those cases in which the head showed enlargement. The generalised swelling of the head, which was noted in two cases, was symmetrical and fairly uniform over the whole of the affected area. Loosening of the teeth was most pronounced in the premolars and canines, although the incisors were also affected.

Microscopic Changes. On the basis of the six cases which were examined histologically, the changes in the individual bones of

the skull will be described in detail. Although there were minor variations from one animal to another the general picture was similar in all cases.

Mandible (*Nos. 21, 24, 27, 29, 32*). In all instances there was very severe resorption of the outer cortex and the only lamellated bone of this layer that remained took the form of isolated islands of irregular shape completely surrounded and encroached upon by fibrous tissue. Sometimes there was still a certain amount of the original lamellated bone left at the inferior edge of the mandible, but in such cases this too showed extensive resorption. Elsewhere the continuity of the outer cortex had been interrupted by the resorption process which had penetrated the compact layer at numerous points so that in many places, the fibrous osteodystrophic tissue in the subperiosteal region was continuous with that in the interior of the bone. The residual lamellated bone showed enlargement of the Haversian canals, which were often invaded by fibrous tissue. The cement lines of the compact bone were usually faintly visible, but in dog No. 29 they were very pronounced. In most instances osteoid borders were inconspicuous. Resorption of the compacta seemed to have occurred from both the periosteal and the endosteal surfaces of the bone, but it was difficult to be certain where the process had started as it was so far advanced. In some instances, the fibrous tissue had invaded the outer surface of the compact bone and had then proliferated in a plane parallel to the surface of the bone producing a "dissecting resorption", and splitting the cortex into layers separated by fibrous tissue. Around the periphery of the remaining compact bone there was active resorption in progress, with Howship's lacunae containing multinucleate osteoclasts. By far the greatest proportion of the mandible was occupied by vascular, connective tissue composed of plump, spindle-shaped cells morphologically similar to fibroblasts. Near the bone islands there were numerous osteoclasts, but these tended to disappear from the areas where all the bone had been resorbed. Scattered through this tissue were narrow, irregularly shaped trabeculae of coarse fibrous texture composed of osteoid or very poorly calcified bone. They often showed a layer of osteoblasts on the surface. These trabeculae tended to occur near the outer parts of the bone on the site of the cortex which had become resorbed, but were not confined to this zone. In several cases there were signs of bone apposition, but on a very much smaller scale than the simultaneous destructive process; some of the cavities excavated in the compact bone were lined by osteoblasts. The interior of the mandible and the fatty marrow were everywhere replaced by vascular fibrous tissue. In many places small haemorrhages had occurred. No cysts were found.

Superior Maxilla (*No. 29, 32*). The same types of changes were present here as in the mandible and were of an equally severe degree. All parts of the bone were affected.

In one animal (No. 32) there was considerable swelling of the bone produced by very active fibrous tissue proliferation. Apart from this change, the histological appearance differed in no way from that seen elsewhere in other cases; there was practically complete resorption of all bone in the superior maxilla, except for a few remaining islets surrounded by osteoclasts. There was formation of osteoid trabeculae and great proliferation of the fibrous tissue. It would appear, therefore, that the question of whether swelling of the bone occurs depends largely upon the activity or otherwise of the fibroblasts.

Parietal (*Nos. 29 and 32*). In both instances there was evidence of resorption. In No. 29, where material from the region of the sagittal crest was examined, resorption was proceeding from the outer surface with dissecting resorption of the outer plate of the bone. The whole process, however, was somewhat quiescent. Active resorption was proceeding in localised areas near the mid line, where, in places, numerous osteoclasts were to be seen. Elsewhere, although both tables of the bone were extremely thin, there was little sign of active osteoclasis, but the outer table showed great irregularity of contour with deep bays filled with collogenous fibrous tissue; only a few osteoclasts were present in these areas. Lying in the fibrous tissue was an occasional osteoid trabecula. The Haversian canals of the cortex were widened and invaded by fibrous tissue. Osteoid borders of normal width were present. In No. 32, where material was taken from the middle of the parietal plate, resorption was occurring from the periosteal surfaces of both the inner and the outer tables. In the plane of section there was a layer of intact cortex between the subperiosteal bone undergoing resorption and the fatty marrow, which itself showed no signs of involvement. The process was a fairly active one, with vascular fibrous tissue and numerous osteoclasts.

Zygomatic Process of the Frontal (*No. 32*). Complete resorption of the bone had taken place and only a thick band of dense collagen remained.

Supraoccipital (*No. 32*). Material from the supraoccipital showed extensive active resorption of both tables of the bone, but there was evidence of some apposition of bone with osteoblastic activity in places.

Angle of the Mandible (*No. 32*). Apart from one or two islands of bone this was largely resorbed and replaced by vascular, fibroblastic tissue. The residual lamellated bone showed well-marked cement lines; normal osteoid borders were present in places.

Changes in the rest of the Skeleton.

Macroscopic Changes. Changes at any point in the skeleton other than the skull have been consistently absent.

Microscopic Changes. Material was taken for section from many parts of the skeleton.

Femur (Nos. 21, 29, 30 and 31). In Nos. 21 and 30, in both of which the shaft was examined in transverse section, no change was noted. Resorption was present, however, in the other two cases, where the distal epiphysis was taken. In both of these (Nos. 29 and 31), there was subperiosteal resorption and invasion of the Haversian canals and marrow spaces by fibrous tissue.

Ilium (Nos. 29, 30 and 31). In all instances there was definite resorption, both superiosteally and in the spongiosa.

Ribs (Nos. 24, 29, 30 and 31). In No. 24 a fracture had occurred at a fairly recent date and a large callus was present at the site. In the parts of the rib distant from the callus, there was dissecting resorption of the compacta and this was more pronounced on the outer aspect of the rib than on the pleural surface. At the site of the callus the original appearance was obscured by the reparative changes. There was abundant formation of osteoid in the callus. It would appear that this was a pathological fracture. In No. 29 there was active subperiosteal resorption in progress and fibrous tissue invasion of the enlarged Haversian canals. In No. 30, there was active and extensive subperiosteal resorption extending circumferentially around the shaft of the rib. Case No. 31 showed a similar appearance.

Radius (No. 31). A transverse section showed active, extensive subperiosteal resorption around the periphery of the shaft with numerous osteoclasts and fibrous tissue.

The surface of the compact bone of the shaft was eroded by a series of "bays". The marrow spaces were invaded by fibrous tissue. The cement lines of the compacta were very distinct in places. In one site, at the periphery of the shaft where a deep bay had been excavated in the bone by the osteoclastic process, there was a cement line in the depth of the bone concentric with the contour of the "bay," possibly suggesting that, following the original resorption at this point, bone had been laid down again partially filling in the cavity; however, no osteoblastic activity was then in evidence. Rows of osteoblasts were to be found lining some of the marrow spaces and also some of the other cavities which had been eroded in the surface of the bone. Numerous osteoid trabeculae were present in the areas undergoing resorption.

Ulna (No. 31). Marked subperiosteal resorption was present here also, and as in the radius, numerous osteoid trabeculae were present in the fibrous tissue.

First Phalanx (No. 30). A first phalanx of the fore limb showed very severe and extensive subperiosteal resorption of the shaft.

Cervical Vertebra (No. 30). This showed active subperiosteal and dissecting resorption of the compacta. The marrow spaces contained fibrous tissue. The processes of the vertebra showed the changes to a much greater extent than the body of the bone.

Lumbar vertebra (No. 24). There was an extensive band of

dissecting resorption just under the surface of a transverse process and invasion of the marrow spaces by fibrous tissue.

From the descriptions which have been given, it is clear that in this condition the bone changes were generalised throughout most of the skeletal system, although they were absent from the shaft of the femur in two cases. From the examination of the bones, the impression was gained that, particularly in the long bones of the limbs and the ribs, the change tended to commence beneath the periosteum. It is of some interest to note that very extensive change may be present in a bone without any clinical sign being evident.

BONE CHANGES IN DOGS WITH CHRONIC NEPRITIS WITHOUT CLINICAL INVOLVEMENT OF THE SKELETON

Since it was clear from the above observations that the presence or absence of bone change cannot be determined by clinical observation alone, the question arose of how long the changes in these animals with Rubber Jaw had been present in a subclinical form, before they progressed to such a degree of severity that, in the head at least, they became clinically manifest. It is very difficult to conceive that the changes which have been described could have arisen as suddenly and as abruptly as would be suggested by the clinical history of bone softening. The question therefore arises: are these changes present in a subclinical form in cases of chronic nephritis not showing clinical bone change, particularly if prolonged, and, if so, at what stage in the course of renal insufficiency do they arise?

To obtain some information on this point, the bones were examined from a number of dogs affected with nephritis without clinical signs of skeletal complications.

As it is clear that the disease process shows a particular predilection for the bones of the jaws and face, these sites have received the greatest attention. In the skull, material for histological examination has been taken from the following standard sites: (i) a cross-section of the mandible including the 4th premolar; (ii) the superior maxilla, including the 3rd premolar; (iii) a cross-section of the angle of the mandible from below the condyle to the anterior border of the bone; (iv) the parietal from near the centre of the plate; (v) the zygomatic process of the frontal. In many instances, portions of bone have also been removed from other parts of the skull, but the above five sites were most frequently examined.

The affected animals were suffering from various stages and degrees of nephritis, ranging from an acute case of a few days' duration to those which showed grossly scarred and fibrosed kidneys where the renal lesion had been present for a considerable but indeterminate period. None of these animals showed the slightest clinical evidence of bone changes. Particular attention was paid to the state of the jaws and the immobility of the teeth. The changes which were observed are summarised in Table I.

TABLE I

INCIDENCE AND DISTRIBUTION OF OSTEODYSTROPHY IN DOGS AFFECTED WITH CHRONIC NEPHRITIS BUT NOT SHOWING CLINICAL EVIDENCE OF BONE DISEASE.

Case No.	*Age (years)*	*Sex*	*Type and degree of nephritis.*	*Wt. of parathyroid tissue per Kg. of body weight.*	*Mandible: angle.*	*Mandible: ramus.*	*Mandible: incisor area.*	*Superior maxilla.*	*Premaxilla.*	*Zygoma*	*Parietal.*	*Ribs.*	*Ilium.*	*Lumbar vertebrae.*	*Scapula.*	*Humerus.*	*Radius*	*Metacarpals.*	*Digits.*
33	6	*M*	Late, severe chronic nephritis.	9·9 mg./Kg.	+++	+++		+++		+++	++								
34	3	*M*	Severe chronic nephritis.	6·8 mg./Kg.				+	+	+*	—								
35	1¼	*M*	Severe acute nephritis.	Not known	—		—		—		—								
36	¼	*F*	Early chronic nephritis.	Not known		+++						++							
37	10	*M*	Late chronic nephritis. Bile duct carcinoma also.	4·4 mg./Kg.		+			+	+* ++	+* ++	—	+* +	—					
38	9½	*M*	Severe late chronic nephritis.	5·0 mg./Kg.	—	+	+		+	+	—								
39	2	*M*	Severe chronic nephritis.	Glands enlarged but not weighed.	—				+	+++	—	++			—	—	—	—	—

+++ Widespread, intense active resorption.
++ Active resorption; moderate extent and intensity.
+ Focal osteoclasis only.
+• ++• Denotes inactive type of osteodystrophy with much collagen present. The plus signs here indicate the extent to which collagen is present.

In view of the diversity of types of renal lesion which were present in this group, these cases will be described individually.

Case No. 33

This was a male cross-bred bull terrier, six years old, known to have been displaying symptoms of nephritis for at least three months prior to destruction; these were much accentuated in the last two-and-a-half weeks of life. The animal was finally destroyed in uraemic coma.

Summary of autopsy findings.

Macroscopic Examination. There was gross uraemic ulceration of the buccal mucosa with no sign of parodontal disease. There was pulmonary oedema; an irregular subpleural haemmorhage was present in the left lung. A small free blood clot was found in the pericardial sac. Two haemmorhagic patches were present in the gastric mucosa and there were occasional irregular patches of congestion throughout the small and large intestines. The kidneys were very firm and their capsules stripped leaving a granular surface. There was reduction in the width of the renal cortex and dilatation of the renal pelvis. There was some prostatic enlargement.

Microscopic examination of the kidneys revealed extensive fibrosis and severe metastatic calcification. Hyaline hypertensive change was present in some of the glomerular tufts. There was epithelial metaplasia and distension of the medullary collecting tubules. The changes were those of a fairly severe chronic nephritis.

Histological examination of the other organs revealed no significant change.

Changes in the bones

The Mandible. The cortex of the mandible was present as a continuous outer casing but, at the same time, showed very pronounced resorptive processes at work which were more severe on the lateral aspect of the mandible. In these areas, there were active osteoclastic resorption, much fibrous tissue and, particularly on the lateral surface of the mandibular ramus, numerous osteoid trabeculae. Some of the latter had osteoclasts in their neighbourhood. The Haversian canals were widened and invaded by fibrous tissue. The inferior edge of the mandible and much of the medial surface showed no subperiosteal resorption, but this change reappeared as the gingival edge of the mandible was approached. Nevertheless, the Haversian canals of the inner aspect of the mandible were widened and invaded by fibrous tissue. Cement lines were not prominent in the compact bone except just under the periosteal surface of the medial and inferior borders of the bone where three or four resting lines, lying close to one another, ran parallel with the surface of the bone. Osteoid borders were present in places, but they were not of abnormal width. The fibroblastic replacement tissue was extremely vascular.

Superior Maxilla. The changes here were very similar to those in the mandible and fully as severe, with resorption of the outer surface of the

body of the maxilla, active osteoclastic resorption and formation of osteoid trabeculae (Fig. 6). There was widespread invasion of the marrow spaces and Haversian canals by fibrous tissue. The same changes were present, in practically the same degree, on the medial aspect of the bone where it bounded the nasal cavity. Although there was much replacement of the marrow by fibrous tissue throughout the whole bone, there still remained a considerable amount of unaffected fatty marrow. Well marked cement lines were present in some parts of the residual bone. Although the outer plate of the maxilla appeared to retain its anatomical continuity, it could be seen microscopically that the resorptive process had lead to numerous perforations of the cortex. It would appear that the structural support which the thinned and perforated bone was able to offer must have been greatly reduced.

Zygomatic process of the frontal. This again showed very extensive resorption which at one site had perforated the bone from one surface to the other. The resorption was active with numerous osteoclasts and fibrous tissue. There was also invasion of the enlarged Haversian canals.

Parietal. Osteodystrophic changes were present in this bone, but the process appeared much less active than in the bones already described. There was resorption of both the inner and outer tables, particularly of the latter. Irregularly shaped "bays" filled with fibrous tissue were present in the subperiosteal region of the bone. The fibrous tissue was less vascular than that found in the jaw bones and, although osteoclasts were present, they were not found in large numbers. Osteoid trabeculae were present in the fibrous tissue of the outer table and there was invasion of the Haversian canals by fibrous tissue. Extensive dissecting resorption of the inner table was present.

Angle of the mandible. There was active resorption of the bone at this site, subperiosteally and in the marrow spaces. The areas undergoing resorption were replaced by young fibrous tissue with numerous fibroblasts and were intensely vascular.

Although there was no sign of bone change macroscopically, histological examination of the skull of this animal leaves no doubt that, in fact, active resorption was in progress and that in some bones at least there must have been a considerable structural weakening as a result.

Case No. 34

A male bull terrier, three years old, with a history of six weeks' illness terminating in uraemia and the clinical signs of severe renal insufficiency. The blood urea was 143 mg. per cent. The dog was destroyed.

Summary of autopsy findings

Macroscopic Examination. There was emaciation and dehydration. The mucosae were anaemic. There was uraemic ulceration of the lower surface of the tongue. Parodontal disease was absent. The pericardium contained several small fibrin clots and there were epicardial ecchymoses. The left auricular endocardium showed a necrotising endocarditis. There was subendocardial congestion of both ventricles. The aorta and pulmonary artery showed no change. There was some pulmonary oedema. The kidneys

were of normal size and their capsules stripped readily, leaving a finely granular surface stippled with minute white points. The bladder and prostate were normal.

Microscopic Examination

The kidneys showed extensive intertubular fibrosis, although many functional tubules still remained. There was a dense, round-celled infiltration in places and calcification of the basement membrane of the tubules and Bowman's capsule. Free collections of calcium granules were present in the capsular spaces of many of the glomeruli. The glomerular tufts showed hyaline, hypertensive changes in patches and sometimes capsular adhesions. The capsular spaces were in places very dilated. The medulla showed epithelial metaplasia of the collecting tubules which occasionally contained masses of polymorphonuclear leucocytes and cellular debris. The arteries showed pronounced hyaline changes.

Changes in the bones

Superior maxilla. The palatine process showed extensive resorption, but the lesions did not appear to be very active. The contour of the bone was very irregular, with the erosions in its surface filled with fibroblastic tissue in which lay trabeculae of osteoid tissue and newly formed bone. Small groups of osteoclasts were present around small islands of bone undergoing resorption. There was little osteoclastic activity on the surface of the residual compact bone. Rows of osteoblasts were present around some of the osteoid trabeculae. The bone bounding the nasal cavity showed little change, apart from a few, small shallow defects filled with fibrous tissue without osteoclasts. The external facial surface of the bone showed the same changes as the palatine process, but osteoid trabeculae were absent. There was active osteoclasis in progress at several points in the interior of the bone, but little fibrous tissue. Cement lines were well marked.

Premaxilla. There were several small subperiosteal erosions in the bone in which active osteoclasis was seen. These foci of resorption were of comparatively small extent compared with the mass of the bone as a whole.

Parietal. No change evident.

Zygomatic process of the frontal. There had been marked resorption of certain areas of the bone, but the process appeared to be inactive for the most part. In these sites the normal continuity of the bone was interrupted by areas of fibrous tissue in which lay osteoid trabeculae. In some of these areas were groups of osteoclasts, but such foci of active osteoclasis were rare.

Compared with case No. 33, the changes in the bones in this dog were less extensive and also of a less active character. They gave the impression that the actual resorption of bone had taken place some considerable time before death, and that the defects had subsequently persisted in a non-progressive state and there had been no restitution of the bone.

Case No. 35

A male Dalmatian, 15 months old. The dog was destroyed in

extremis eight days after the onset of acute nephritis. There was no history of any earlier renal disease. The blood urea at death was 369 mg. per cent.

Summary of autopsy findings

There was extensive necrotising endocarditis of the left auricle and a small patch of necrotising arteritis in the pulmonary artery. The kidneys were swollen and congested. The parathyroids were not noticeably enlarged. Microscopically the kidneys showed patchy acute interstitial nephritis becoming confluent near the boundary zone.

Changes in the bones

No evidence of bone resorption could be found in the parietal, premaxilla or mandible (Incisor area or region of the angle).

Case No. 36

A female bloodhound, three months old, which had shown evidence of renal damage for at least six weeks before death. The post-mortem examination was performed by T. Pay, B.Sc., M.R.C.V.S., of the Royal Veterinary College, to whom I am indebted for the details of the autopsy, and for the kidneys, parathyroids, and samples from the bones.

Summary of autopsy findings

At autopsy, the kidneys showed some fibrosis and capsular adhesions. The parathyroids were enlarged. Microscopically, the kidneys showed localised areas of scar tissue and some metastatic calcification. There was extensive destruction and distortion of glomeruli. The changes were those of early chronic nephritis.

Changes in the bones

Mandible. This bone showed extensive resorption with many osteoclasts and the enlarged Haversian canals were filled with fibrous tissue. Some trabeculae showed evidence of osteoblastic activity. There was slight thinning of the predentine of an erupting permanent premolar.

Costo-chondral junction. The bone of the rib distant from the cartilage showed several areas of active resorption with numerous osteoclasts and fibrous tissue, and also some osteoblastic activity around the trabeculae. At the epiphysis, there was a marked widening of the zone of hypertrophic cartilage cells, compared with that of a normal dog of about the same age (Figs. 8 and 9). Osteoclasts were very numerous in the zone of calcified cartilage and there was marked increase in fibrous tissue in the marrow spaces. There was no sign of excessive osteoid production.

This case provides a good example of the effects of chronic renal insufficiency on the growing skeleton. There was evidence of retardation of growth and rapid breakdown of newly formed bone.

Case No. 37

A male Sealyham, ten years old. The dog was destroyed following a clinical diagnosis of an hepatic neoplasm and chronic nephritis. The terminal blood urea was 214 mg. per cent.

Summary of autopsy findings

Macroscopic Examination. There was emaciation, anaemia and slight icterus. Free blood (50 cc.) was present in the peritoneal cavity. The colon showed a band of deep congestion of the mucosa and there was a large hepatic neoplasm. The lungs were oedematous. The kidneys were normal in size; their capsules stripped readily leaving a slightly granular surface. The right adrenal contained a cortical adenoma. The other organs were macroscopically normal.

Microscopic Examination. The liver tumour was a well-differentiated carcinoma of bile duct origin with metastases in the small vessels of the lungs and in the hepatic lymph nodes. The kidney showed fibrosis of irregular distribution becoming confluent near the boundary zone. In places there was severe hyaline arteriolar change, and patches of hyaline were present in the glomerular tufts. There was no metastatic calcification. Metaplasia of the epithelium of the collecting tubules and medullary inter-tubular fibrosis were present. No secondary deposits were found in the kidney.

Changes in the bones

Premaxilla. This bone showed resorption in occasional small foci of osteoclasts on the surface and in the cancellous bone. Elsewhere there were irregular defects in the contour of the bone surface filled with collagenous fibrous tissue.

Mandible. There was active subperiosteal resorption in the incisor part of the bone, but the erosions thus formed were very shallow and there was no narrow fibrosis. Elsewhere in the mandible there was little change apart from some osteoporosis.

Zygomatic Arch. There was a large area of inactive osteodystrophy which traversed the whole width of the bone. In this area, the bone had been largely resorbed and replaced by a collagenous fibrous tissue not very rich in cells, into which residual trabeculae of bone protruded. No osteoclasts were present.

Parietal. This bone also showed a large area of inactive osteodystrophy in which there was replacement of the bone and the marrow spaces by collagenous fibrous tissue. Osteoclasts were not in evidence.

Rib. No obvious changes were detected.

Ilium. There was an area of inactive osteodystrophy of the same type as in the other bones.

Lumbar vertebrae. This bone showed no change.

The bone changes in this animal were of a considerably less active type than in the instances described hitherto. At some time there had apparently been an active resorptive process which had later become quiescent. The defects in the bone persisted with the formation of dense collagenous fibrous tissue. Only small and occasional foci of active osteoclasis were present.

Case No. 38

A male smooth-haired terrier nine-and-a-half years old. There was a history of chronic nephritis extending over "several" months

and the dog was finally destroyed in a state of clinical uraemia. The blood urea level at death was 255 mg. per cent.

Summary of autopsy findings

There was ulceration of the tongue and buccal mucosa and some patchy congestion in the stomach and small intestines. The liver showed several plexiform haemangiomata. There were some splenic-omental adhesions. No change was noted in the respiratory system or the heart. The kidneys were reduced in size, wrinkled and fibrous. The prostate was somewhat enlarged. Each adrenal contained a small cortical adenoma. Histologically, the kidneys showed extensive replacement fibrosis and scarring.

Changes in the bones

There were small localised foci of resorption in the premaxilla, the mandible (in the incisor region), in the horizontal ramus of the mandible and in the zygoma. No change could be detected in the parietal nor in the mandible in the region of the angle of the jaw.

Case No. 39

A male Alsatian, two years old. There was a history of acute leptospirosis at the age of ten months. Symptoms of residual renal damage persisted for some months and then subsided. Subsequently they recurred and the dog was destroyed a few days later in a state of severe renal insufficiency.

Summary of autopsy findings

There was ulceration of the buccal mucosa and a deep congestion of the stomach. The respiratory system showed no change. Extensive healed lesions of necrotising endocarditis and arteritis were present. The kidneys showed a granular surface and an increase in fibrous tissue.

The parathyroids were enlarged, but were not weighed. Histologically, the kidneys showed extensive diffuse interstitial fibrosis and widespread hyaline hypertensive changes in the glomeruli and arteries.

Changes in the bones

Rib. There was some subperiosteal resorption of moderate degree along the shaft of the rib. The process was active with osteoclasts and fibrous tissue replacement.

Premaxilla. This bone showed a well marked but localised resorption in the alveolus of an incisor tooth.

Zygomatic arch. There was a marked active resorption which at one point had lead to perforation of the bone.

No resorptive changes were present in the scapula, metacarpals, digits, humerus, the radius, the angle of the mandible and the parietal bones.

The changes in this case were of slight degree only, but there were several sites in which localised, but active, resorption was in progress.

It is evident that there is considerable variation in the intensity of the osteodystrophic process which is present in chronic nephritis. Of the six cases of chronic nephritis which have been examined for histological bone changes, three have shown slight lesions only while three have been more severely affected. The possible reasons for these variations will be discussed later. The changes, however, are qualitatively similar to those seen in Rubber Jaw.

DISCUSSION

The disease known as Rubber Jaw is a condition in which there is a widespread osteodystrophia fibrosa throughout the skeletal system, while in the head the change is so extensive that its effects are manifest clinically. The lesions in the bones are associated with a severe chronic nephritis and enlargement of the parathyroids. It has been shown that changes of a similar kind are present also in cases of chronic nephritis in which there is no outward sign of skeletal abnormality. Indeed, even in an early case of chronic nephritis (No. 36), every feature of the fully developed condition as seen in Rubber Jaw, was represented though on a less extensive scale. In view of the marked similarity between the bone, parathyroid and kidney changes in Rubber Jaw and in chronic nephritis, it seems likely that the two conditions are closely related. Some enlargement of the parathyroids was a constant feature of both, and from the rather limited evidence available there seemed to be some corelation between the extent of this and the severity of the bone lesions.

Bone changes of the same type as those in Rubber Jaw were consistently present also in chronic nephritis, although in several cases of the latter they were only slight in degree and localised in extent: the difference, however, was purely quantitative. It seems probable therefore that Rubber Jaw is a condition in which the bone changes which are frequently seen in chronic nephritis have reached such a degree of severity, that their effects have become clinically manifest. It seems unlikely that in Rubber Jaw the parathyroid enlargement is primary and the renal disease is secondary, particularly since metastatic calcification of the kidneys was either very slight or completely absent in several examples of the condition (Platt, 1951b).

The changes in the bones which are evoked by a prolonged state of renal insufficiency depend upon the state of maturity of the skeleton. In a young animal, such as No. 36, the effects of chronic renal impairment during an active growth period have resulted, in addition to the osteodystrophic changes found in adult dogs, in a retardation in the epiphysial ossification and formation of bone. On the other hand, in the adult skeleton, there is a considerable variation from one animal to another in the severity of the resorptive process which the renal insufficiency has excited. Several

variable factors may be responsible for this, such as the calcium intake, the severity and duration of renal insufficiency and possibly the degree of parathyroid enlargement. The degree of renal functional compensation which takes place following the initial damage to the kidneys varies from one case to another and histological examination alone is probably inadequate to assess this factor. The nature of the diet also probably influences the severity of the resorptive process and diets which are low in available calcium and rich in foods likely to be broken down to acid end-products would tend to aggravate the process. Jaffe, Bodansky and Chandler (1932) showed experimentally that a state of chronic acidosis, particularly if associated with a low calcium intake, may give rise to osteodystrophic changes in the skeleton particularly in young dogs. Whether or not this would apply to adult dogs is not clear, however, but it may possibly play a contributory part in the pathogenesis of osteodystrophy. The development of actual clinical bone changes can only be expected when all these factors and possibly others favour a severe and progressive breakdown of bone with inadequate apposition to compensate for it. It would certainly appear that in the jaw, for example, as long as a thin, outer shell of compact bone is preserved, the rigidity of the part is maintained even though very severe resorptive change is present in the cancellous and compact parts of the bone. Once the amount of compact bone is reduced below a certain critical minimum, however, impairment of the normal rigid support provided by the bone may be expected.

A feature of Rubber Jaw which is more difficult to explain is the great predisposition shown by the jaws and bones of the head. It has been seen that if bones from other parts of the skeleton are examined, the osteodystrophic condition is found to be generalised. For some reason, however, the jaws are the site of most intense change. The reason for this is not clear, but it is relevant to note that this part of the skeleton is a predilection site for osteodystrophia fibrosa in other animal species, such as the horse, goat and pig. It may be that this part of the skeleton has a greater lability than elsewhere; if resorption and apposition normally occur more rapidly here, any process which tended to increase resorption and impair apposition of bone would have more pronounced effects than in any other place.

Weber and Becks (1932) have attempted to produce osteodystrophia fibrosa experimentally in dogs by feeding them a diet which was very low in calcium, with a wide Ca : P ratio and free from Vitamin D. There were ample opportunities for solar irradiation so that the effect of the Vitamin D deficiency was rather an imponderable factor. Typical changes developed in the jaws, which showed well marked osteodystrophy. Less severe changes were present in other parts of the skeleton. It was concluded by the authors that the diet was responsible, but it is possibly signifi-

cant that, of the eleven dogs which were employed at the outset of the experiment, five died soon afterwards and two more after they had been fed on the diet for some time; all these animals died from nephritis or bronchopneumonia. It may well be, therefore, that the four animals on which the writers based their conclusions had also sustained kidney damage from which they had partially recovered. They made no comment on the state of the kidneys. The bone changes were also described in the two animals which died during the course of the experiment. One of these had been on the diet for six weeks and died of nephritis; the jaws showed well-marked osteodystrophia fibrosa. The other had been on the diet for seven months before dying from bronchopneumonia. The bones in this case showed osteoporosis only. It would appear that the state of the kidneys was a more potent factor in determining the development of osteodystrophy than calcium deficiency alone, judging from these two cases. However, there seems little doubt that a state of reduced calcium intake tends to render the skeleton much more susceptible to the effects of resorptive changes but whether calcium deficiency or a wide Ca:P ratio can produce osteodystrophia fibrosa in the dog without the intervention of other factors is not altogether clear.

A survey of the literature reveals a number of instances of bone disease in the dog which on histological grounds justify the diagnosis of osteodystrophia fibrosa, but the exact pathogenesis of the majority of them is quite obscure since in none of them, except the case of Perlmann (1944), are the parathyroids described. In many instances the kidneys are not mentioned either. It is probable that cases such as those of Schmey (1915) and the second case of Pallaske (1934) are examples of Rubber Jaw. The other type of canine osteodystrophy which is mentioned as occurring in young dogs and clinically, simulating rickets, appears to be of a different nature. However, without reference to the kidneys and parathyroids it is difficult to form any opinion as to its etiology.

SUMMARY

It has been shown that the bone lesions in Rubber Jaw are those of an osteodystrophia fibrosa and that, although the head is the most severely affected site, lesions of a similar type are present throughout most of the skeleton.

The results of histological examination of the skeletal system of seven dogs affected with various stages and degrees of nephritis are described. Three of these animals showed well-marked osteodystrophic changes in spite of the fact that the skeleton appeared clinically normal. The other four showed similar changes of a lesser degree.

The relation of the Rubber Jaw syndrome to chronic renal insufficiency is discussed together with a review of some of the factors which appear to be of importance in the pathogenesis of canine osteodystrophy.

ACKNOWLEDGMENTS

I should like to express my deep appreciation of the advice and help which I have received from Professor G. Payling Wright during the period in which this work was carried out and also for his criticism of this paper. I should also like to thank Professor M. A. Rushton, of the Department of Dental Medicine, Guy's Hospital, for his help and advice in the interpretation of the bone sections. I am indebted to Messrs. J. F. C. Wilder, R. J. Morgan and L. Till for their technical assistance, and to the Agricultural Research Council and Animal Health Trust for grants, and to Miss J. O. Joshua, F.R.C.V.S., and other veterinary surgeons who sent me cases for study.

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CAPTIONS

FIG. 2. Mandible. Rubber Jaw showing osteodystrophic tissue. Note the involvement of the cementum of a neighbouring tooth. H. and E. × 114.

FIG. 3. Radius. Rubber Jaw. Note the severe subperiosteal resorption. H. and E. × 60.

FIG. 4. Ulna. Rubber Jaw; Active subperiosteal resorption in progress. H. and E. × 60.

FIG. 5. First Phalanx. Rubber Jaw. Severe resorption. H. and E. × 60.

FIG. 6. Superior maxilla. Chronic nephritis. Case No. 33 shows active bone resorption. No clinical evidence of bone softening was present in this animal. H. and E. × 118.

FIG. 7. Zygoma. Chronic nephritis. No. 37. Inactive form of osteodystrophy. Note the dense collagen and absence of osteoclasts. H. and E. × 60.

FIG. 8. Costo-chrondral junction of a normal 3 month-old dog. H. and E. × 60.

FIG. 9. Costo-chondral junction of a three-month old dog with a six weeks' history of nephritis (Case No. 36). Note the widening of the zone of hypertrophied cartilage cells. The edge of the zone of the zone of provisional calcification is irregular. There is some increase in the number of osteoclasts. H. and E. × 60.

Fig. 2

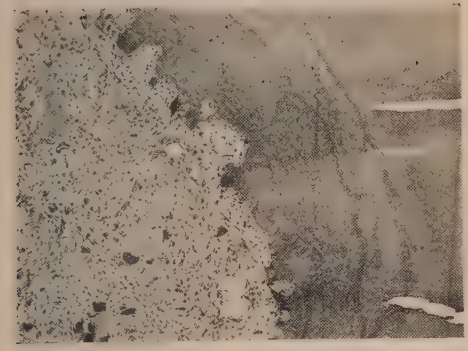

Fig. 3

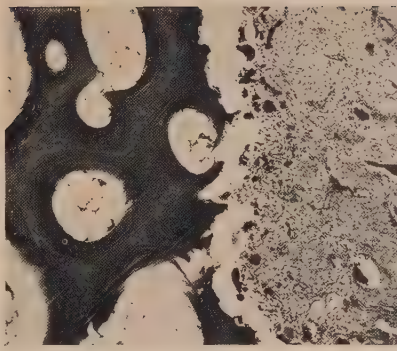

Fig. 4

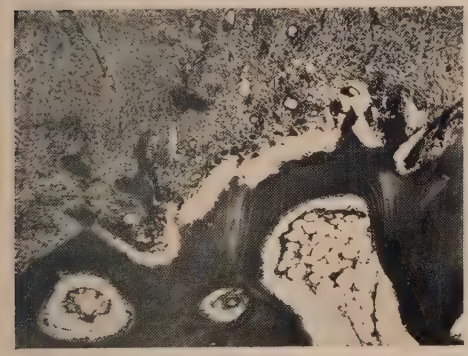

Fig. 5

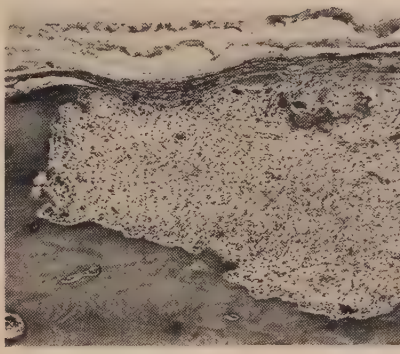

Fig. 6

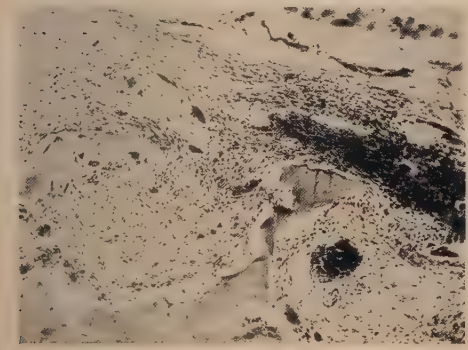

Fig. 7

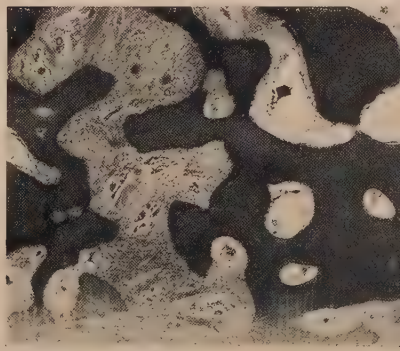

Fig. 8

Fig. 9

DEVELOPMENTAL FORMS OF THE VIRUS OF OVINE ENZOOTIC ABORTION

By

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INTRODUCTION

Stamp, McEwen, Watt and Nisbet (1950) described a placentitis of the ewe caused by a virus showing morphological similarities to the elementary bodies of the psittacosis-lymphogranuloma group of organisms. Cultures of the virus obtained fairly easily in the yolk sac of a six or seven day chick embryo which is killed in 5 to 8 days. The virus also grows quite readily on the chorio-allantoic membrane, but in this case the embryo remains viable. In recent years investigations by a number of workers have shown that there is a group of viral agents which is morphologically similar to the viruses of psittacosis and lymphogranuloma venereum in that they form elementary bodies and appear to undergo a definite life cycle showing various developmental forms. The proper classification of the virus under study as a member of this group is thus, in part, dependent upon the demonstration of similar developmental forms.

MATERIAL AND METHOD

In order to demonstrate the possible presence of these developmental forms 0·25 cc. of a 10 per cent. broth suspension of a heavily infected yolk sac was inoculated on to the chorio-allantoic membrane of thirteen-day-old chick embryos using the technique described by Beveridge and Burnet (1946). In the first instance two eggs were killed and examined every two hours after inoculation but later it became obvious that the period 34 to 48 hours after inoculation was the most informative period so that eggs were examined more frequently during these hours of growth. Impression preparations of the infected membrane were made by cutting about one square cm. of membrane and laying the cut piece, ectodermal surface down, on to a clean dry slide. The cut membrane was then blotted and the slide warmed in the paraffin oven. When almost dry the blotting paper was lifted off, carrying most of the membrane with it, but leaving behind on the slide a good deal of ectoderm. The impression smear was then fixed in methyl alcohol, stained by prolonged Giemsa and afterwards differentiated in alcohol or on occasion acetone. Other pieces of the membrane were taken for histological examination, fixed in Regaud's fixative and sections stained by Giemsa.

Examination of the Chorio-allantoic membrane

The most characteristic lesions are seen in eggs opened 3 to 5

days after inoculation, although the macroscopic lesions are never very significant. The membrane may be thickened by oedema and show a varying degree of general opacity, there may be flecks but sharply defined lesions are not seen. The histological appearance of such a membrane is characteristic in that on the surface of the epithelium are darkly staining virus colonies packed with virus bodies. These colonies are surrounded by a degenerating zone of epithelium staining a homogeneous pink colour. The epithelial cells proliferate and are vacuolated to some degree, but the changes in the mesodermal and ectodermal layers are insignificant unless the membrane has undergone mechanical trauma (Fig. 1 and 2).

Growth behaviour of the virus

Some 36 hours after inoculation there appear virus bodies 0·5 to 0·7 μ in diameter staining deeply with the basic dyes and generally spherical in shape (Fig. 3). Within a short time these bodies grow in number and in size to become large intensely staining structureless bodies of 6 to 8 μ in diameter. Even after prolonged differentiation the structureless nature of the matrix remains except that at the periphery of some plaques it was possible to make out collections of darker staining bodies almost hidden by the matrix (Fig. 4). At slightly later stages the plaques begin to lose some of their affinity for the basic dye especially at the periphery and in the paler matrix one can now see the development of elementary bodies (Figs. 5 and 6). A number of plaques appear to become granular early in their formation and differentiate rapidly and evenly into colonies of elementary bodies without the prior formation of the large deeply staining bodies within the matrix of the plaque (Fig. 7).

A further method of development is, however, seen alongside the formation of the plaques. The initial virus bodies which appear at about 36 hours, instead of developing into plaques, may proliferate as such to form colonies of large forms of virus without any definite matrix; they are circular, oval or angular with flattened sides. These large forms do not stain well with the basic dye and not infrequently the bodies take on different depths of colour in the same colony. These virus colonies grow considerably in size, the large forms multiplying in number but at the same time becoming smaller in size to form colonies of intermediate forms (Figs. 8, 9, 10, 11, 12, 13 and 14). Finally by 72 hours the bodies have decreased in size to such a degree that the colonies are now packed only with elementary bodies.

It would also appear that the elementary bodies can divide as such to form colonies.

DISCUSSION

The virus of ovine enzootic abortion would appear to have a developmental cycle similar in many ways to that of the viruses of psittacosis and lymphogranuloma venereum. For descriptive pur-

poses the development of the virus falls into several phases but these go on side by side in the same eggs and at the same time intervals. The phases may be classified as follows:

1. Homogeneous plaques which may develop into large forms and then into colonies of elementary bodies.

2. Smaller plaques which develop rapidly and directly into colonies of elementary bodies.

3. Colonies of large and intermediate forms without the prior formation of a plaque. These develop into colonies of elementary bodies.

4. Colonies of elementary bodies multiplying as such.

The present work adds nothing further to the previous interpretations of the virus plaque (Bedson and Bland, 1934; Bland and Canti, 1935; Levinthal, 1935; Yanamura and Meyer, 1941; Rake and Jones, 1942). The findings are very similar to those previously described in that it would appear that the homogeneous plaque is a colony of large forms obscured by an extremely dense matrix. The origin of these forms in the plaque cannot be determined since the smaller plaques remain completely homogeneous even after very prolonged differentiation. The second type of plaque which differentiates early into colonies of elementary bodies suggests very strongly that the elementary bodies have been merely hidden by a matrix and that the plaque in this case is more in the nature of an inclusion body. The two methods of development of the plaque appear very different.

The colonies of large forms on the other hand are very definite having no confusing matrix. The evidence here is very clear that the large forms develop directly and multiply as large forms, becoming in turn intermediate forms and finally finishing as a virus colony of elementary bodies. In this form there is no confusion with matrix and the appearance of the colonies suggests that the large forms are virus material and not inclusion bodies of cytoplasmic matrix.

SUMMARY

The development of the virus of ovine enzootic abortion on the chorio-allantoic membrane of the chick embryo is described.

The findings suggest that this virus belongs to the psittacosis-lymphogranuloma group.

ACKNOWLEDGMENT

I am grateful to Mr. R. Cockburn for his help in the preparation of the material and the photographs.

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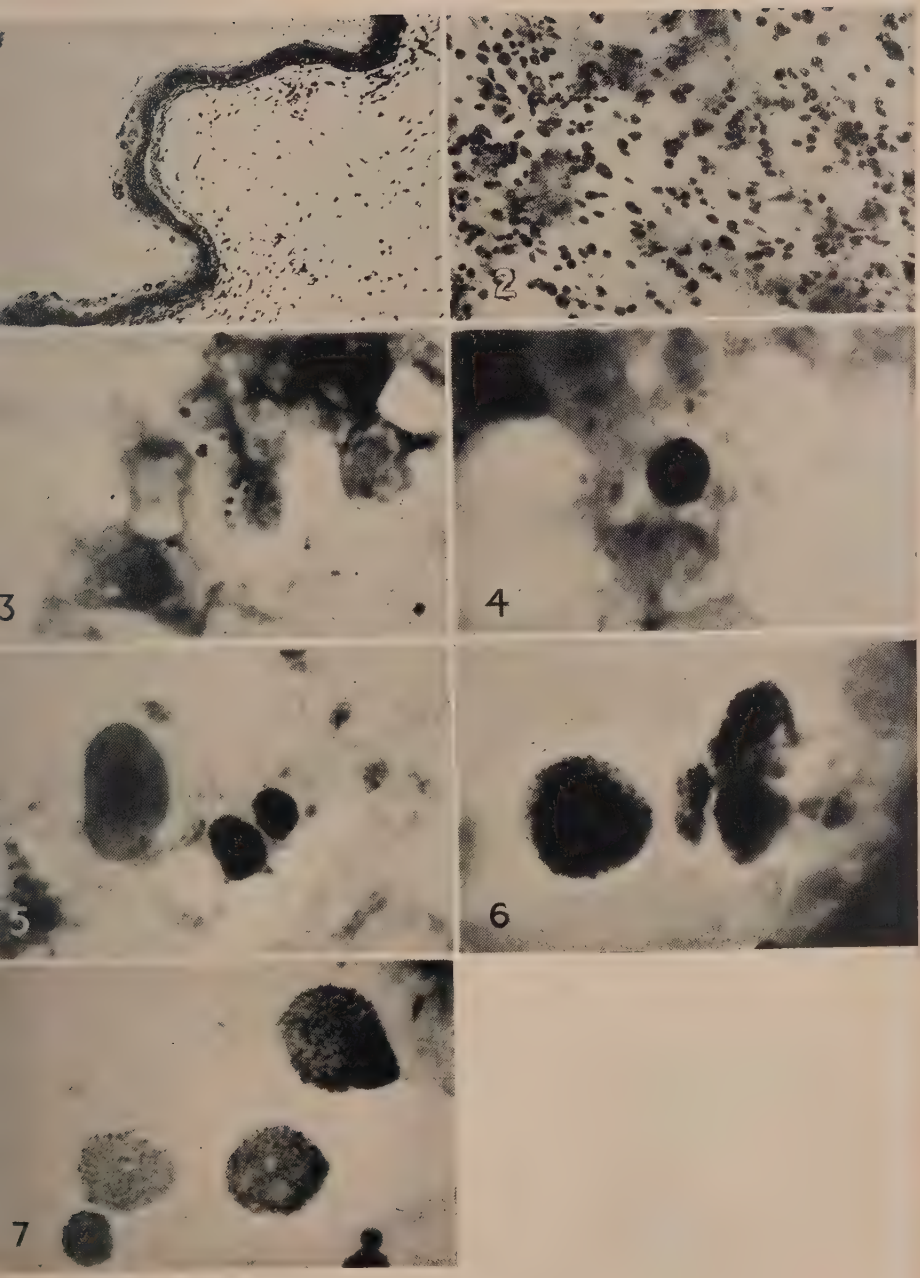
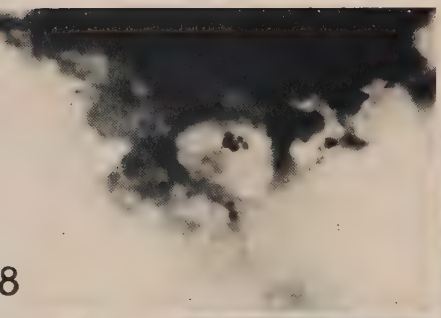


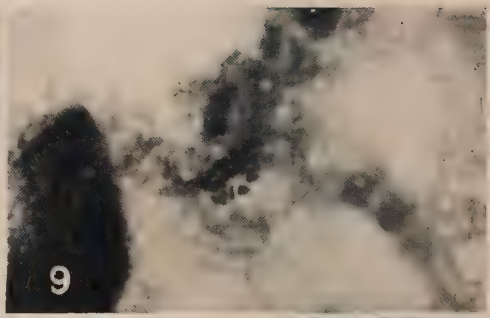
Fig. 1. Section of chorio-allantoic membrane showing the virus colonies of elementary bodies on the surface. Giemsa × 100.

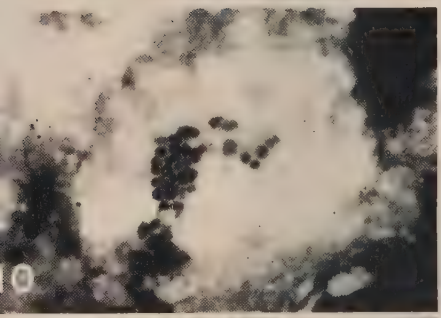
Fig. 2. Impression smear of chorio-allantoic membrane showing large numbers of virus colonies packed with elementary bodies. Giemsa × 100.

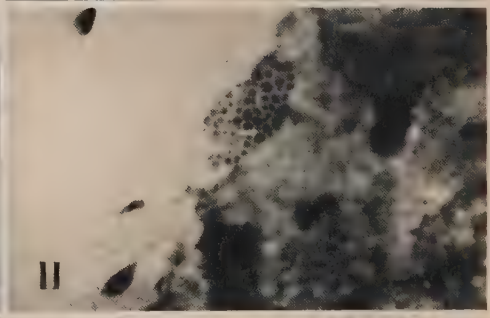
Fig. 3. Impression smear of chorio-allontoic membrane showing the first virus bodies to be seen 36 hours after infection. Giemsa × 1,500.

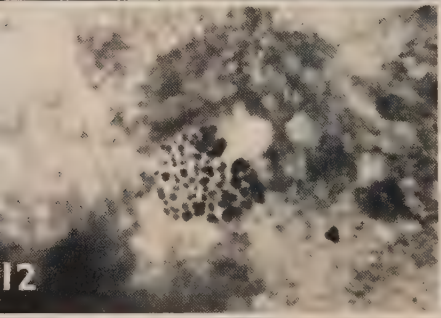
Figs. 4, 5, 6 & 7. Impression smears of chorio-allantoic membranes showing successive stages in the development of elementary bodies from the structureless plaque. Giemsa × 1,500.

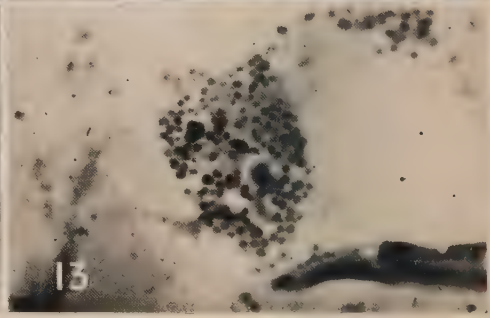


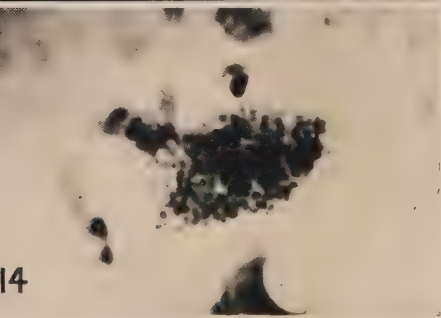












Figs, 8, 9, 10 & 11. Impression smears of chorio-allantoic membranes showing various stages in the development of colonies of large forms of virus. Giemsa × 1,500.

Figs. 12, 13 & 14. Impression smears of chorio-allantoic membranes showing the formation of colonies of intermediate forms and elementary bodies arising from the colonies of large forms of virus. Giemsa × 1,500.

PATHOLOGICAL AND SEMINAL CHANGES IN BULLS AFFECTED WITH A SPECIFIC VENEREAL INFECTION

By

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INTRODUCTION

A prevalent and serious type of bovine sterility in Kenya is caused by a specific venereal infection (Daubney, Hudson and Anderson, 1938; Anderson, 1949; Hudson, 1949). In this communication a study of the pathology of the condition in the bull is reported and a comparison is made of the pathological changes in the genital organs and those previously noted (Anderson, 1951) in the physio-chemical composition of the semen of affected bulls.

MATERIAL AND METHODS

Three pedigree Friesian bulls from a herd affected with the disease had clinical lesions of epididymitis when first seen in August 1948. They were under observation until February 1949. Bulls Nos. 1 and 2 had bilateral epididymitis and No. 3 unilateral epididymitis.

Portions of the following organs were fixed in 10 per cent. formol-saline and sectioned after paraffin embedding: prostate gland, seminal vesicle, ampulae, vas deferens, cauda epididymis (together with the caput epididymis in the case of Bull No. 1) and the central part of the testis. Staining methods employed were haematoxylin and eosin and Mason's haematoxylin-Ponceau-acid fuchsin—light green. Subsequent operations have shown that Giemsa's stain gives a better picture of the interstitial infiltrations which occur in this disease.

Semen was collected in the usual way in an artificial vagina. Sugar was estimated either by Hardin's modification of Schaffer-Hartman method or by Haslewood and Strookman's method (King, 1946). The methods used for determining the buffering capacity (B.C.) and the specific gravity (S.G.) have already been described (Anderson, 1946a).

EXAMINATION OF THE SEMEN

The results of the examination of semen are shown in Table I. During the period of observation the *pH* decreased in Nos. 1 and 3, but still remained alkaline; the fructose content increased to a normal figure as did the specific gravity. The buffering capacity improved in No. 3 but was still high in No. 1. Bull No. 2, on the other hand, showed a deterioration in all criteria over the seven month's period.

TABLE I

Bull	*Date*	*pH*	*Fructose Content (Mg/100ml semen)*	*Buffering Capacity*	*Specific Gravity*	*Spermatozoa*
No. 1	14.8.48	7·56	182	—	—	
		7·80	177	—	—	
	30.9.48	7.90	60	2·50	1·0147	Absent
	15.2.49	7·70	512	2·00	1·0294	,,
		7·30	727	2·60	1·0327	,,
No. 2	23.7.48	7·64	427	1·68	—	Absent
	13.8.48	7·29	917	2·00	1·0281	,,
		7·31	905	2·80	1·0270	,,
	30.9.48	7·68	613	1·48	1·0331	,,
	15.2.49	7·80	36	3·92	1·0112	,,
		—	123	3·37	1·0078	,,
No. 3	30.8.48	7·50	0	3·18	1·0230	260,000/cmm.
	30.9.48	8·22	18	·278	1·0114	Absent
	15.2.49	7·08	757	2·08	1·0334	,,
		7·28	786	2·22	1·0341	,,

OBSERVATIONS AT POST-MORTEM EXAMINATION

Post-mortem material examined consisted of the whole genital tracts collected at the abattoir immediately after slaughter of the three bulls.

Bull No. 1

Right testis. The seminiferous tissue was reduced and measured $2 \times 2\frac{1}{2} \times 2\frac{1}{2}$ in. so that it was less than a quarter of the organ. Large fibrous enlargements of the caput and the cauda of the epididymis were present, both measuring $2 \times 2\frac{1}{2} \times 2\frac{1}{2}$ in. and were joined together by a thick mass of connective tissue representing the corpus epididymis. The tunica vaginalis reflexa was attached by strong fibrous adhesions to the testis.

Left testis. The seminiferous tissue measured $2\frac{1}{2} \times 1\frac{1}{2} \times \frac{1}{2}$ in. The epididymis had changes similar to those found in the right testicle. The tunica vaginalis reflexa was attached as in right testicle. The right vesiculae seminales measured $4\frac{1}{2} \times 1\frac{1}{2} \times 1$ in. and was firm; the left, 4×1 in. was almost tubular in shape. The ampullae were $\frac{1}{2}$ in. in diameter.

Bull No. 2

Right testis. The seminiferous tissue measured $3\frac{1}{2} \times 2\frac{1}{2} \times 1$ in. One large fibrous lesion $3 \times 2\frac{1}{2} \times 1\frac{1}{2}$ in. was present at the tail and the whole body of the epididymis was thickened and band-like, with a width of $1\frac{1}{4}$ in. In the mass at the cauda was a thick,

tenacious, yellow pus. No trichomonads were seen but recognisable spermatoza with rare, live individuals and amorphous debris were present in the fluid. This material also filled the vas deferens. The tunica vaginalis reflexa was strongly attached as in Bull No. 1.

Left testis. The changes in the left testis were less marked. The seminiferous tissues measured $4\frac{1}{2} \times 3 \times 2$ in. A large, egg-shaped, hard fibrous mass represented the tail of the epididymis. On cutting into this many lakes containing pus were present. No trichomonads were seen. The tunica vaginalis reflexa was attached as in Bull No. 1. The vesiculae seminales were large ($5 \times 1\frac{1}{2}$ in.), roughly cylindrical, lobulated and firm. The ampullae were $\frac{3}{4}$ in. in diameter, and were cylindrical.

Bull No. 3

Right testis. The seminiferous tissue measured $5 \times 3\frac{1}{2} \times \frac{3}{4}$ in. The head of the epididymis, which measured $1\frac{1}{2} \times 1 \times \frac{3}{4}$ in., was fibrous and there was a large quantity of semen in the tubules. Many spermatozoa were present of which about 20 per cent. were alive, together with much cellular debris. No trichomonads were seen. The tunica vaginalis reflexa was adhering in patches.

Left testis. The seminiferous tissue was $5\frac{1}{2} \times 3 \times 2\frac{1}{2}$ in. The epididymis measured $2 \times 1 \times 1$ in., and its cut surface was fibrous. On incision, dilated tubes exuding material similar to that recorded for the right testis were seen. The vesiculae seminales were firm and lobulated. The ampullae were firm, turgid and $\frac{3}{4}$ in. wide.

HISTOLOGY

Prostate

In Bull No. 1 the prostate presented an apparently normal histology. In Nos. 2 and 3 there were a few minute interstitial foci of mononuclear infiltration mainly of a lymphocytic type. There were no associated epithelial changes even where these foci occurred immediately subjacent to the secretory cells.

Vesiculae Seminales

The unaffected secretory epithelium appeared to consist of at least three types of cell, the great majority being tall columnar elements with the nucleus situated in the basal third. These cells had an acidophilic granular cytoplasm and did not stain very heavily. There appeared to be no distinct basement membrane, but the free border was well marked and, where seen perpendicularly, polygonal in outline. It stained intensely and, as stated by Macklin and Madge (1932), simulated a cuticular border. The secretory cells were normally separated from the material in the lumina by spherical or elongated clear spaces, probably representing an alcohol or water-soluble secretion.

Interspersed among these cells, with varying frequency, were a few darker, laterally compressed elements. In sections stained by Masson's trichrome method, the nuclei were small, more darkly

stained and somewhat obscured by the intense reddish-grey reaction of the cytoplasm. These cells also extended from the lamina propria to the lumen, where an irregular protrusion of reddish, homogeneous material suggested a discharge of secretion. It is possible that such cells represented a stage in the secretory cycle.

The third cell type present was basal in position, being normally rounded with a nucleus lying close to the subepithelial connective tissues. Sometimes the cytoplasm appeared to be the same as that of the adjacent, larger elements but frequently the cell body formed one or two clear vesicles, the nucleus being pushed to the periphery and flattened. Various stages in the transformation could be seen. Frozen sections of vesicles from other animals show that these vacuoles contain lipoid. The secretion was normally homogeneous and acidophilic. Most of the acini were filled by its accumulation.

In bull No. 1 no abnormality was found in the sections examined but as the examination of one block only is not a reliable method of determining the presence or absence of infection in so complex an organ, the observations on this animal have accordingly a very limited value.

In bull No. 3 the following pathological changes were noted. The secretion was absent in some apparently normal acini and in all those in which inflammatory changes were marked. There was a rather diffuse, cellular infiltration in parts of the gland, particularly those surrounding the larger "ducts". The infiltration was composed largely of mononuclear cells, the majority being epithelioid in character, while some were of a lymphocytic or plasma-cell type together with fibroblasts in various stages of differentiation. The result was a thickening of the "interlobular" septa, sometimes of a focal character though usually diffuse, with a tendency to a linear arrangement of the infiltrating cells (Fig. I). Polymorphonuclear leucocytes were rare except where there was an extension of the inflammatory process into the epithelium overlying an interstitial infiltration, an observation which appears to be true throughout the genital tract in the three cases studied.

Where the epithelium was affected, it was composed of several irregular layers of cells, not characteristically columnar. The boundary of the epithelium was obscured by the cellular infiltration; the individual cells, especially the superficial ones, showed various degenerative changes including chromatolysis, peripheration and clumping of chromatin, desquamation and invasion by leucocytes. Sometimes there was a definite neutrophilic or basophilic body formed within the degenerating nucleus but nothing sufficiently distinct to call an inclusion body was observed.

The most characteristic change was found in the cytoplasm of affected cells which contained distinct round eosinophilic globules. They were refractile and occurred mainly on the distal side of, and surrounding the nucleus (Fig. II), but were by no means invariably

FIG. 1

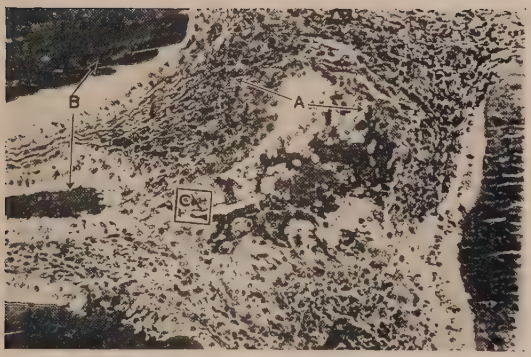


FIG. 2

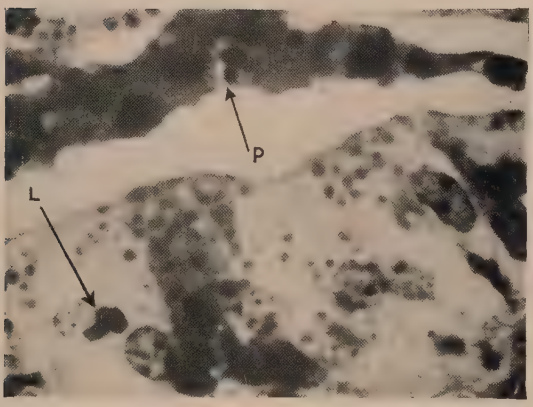
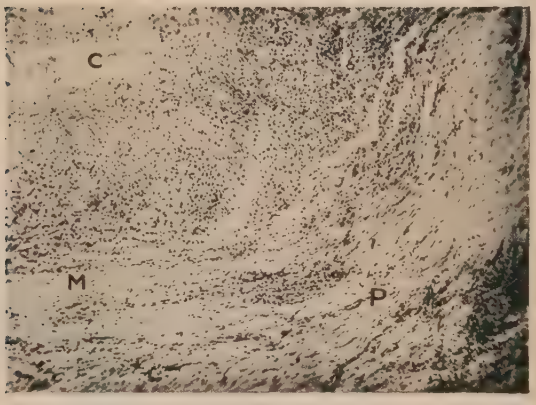


FIG. 3

FIG. 4

FIG. 5

FIG. 6



present. These globules varied greatly in size, and some of the smaller ones were often greenish in preparations stained by Masson's method. Sometimes they completely filled the cell or were scattered when it was disrupted. In the latter case they did not persist long in recognisable form in the lumen. Alternatively only one or two large globules were seen in an otherwise normal cell. The more superficial bodies were usually larger and in this respect resembled secretion granules, but they were not present in the normal epithelium of bulls known to be affected with the specific venereal infection.

Bull No. 2. In this animal the involvement of the seminal vesicles was of a more severe character, as evidenced by the marked changes in the secretion in the majority of acini. These included large numbers of cells, many still recognisable as polymorphonuclear leucocytes. Some acini, especially towards the periphery, contained desquamated epithelial cells in which the acidophilic granules, already described, were visible when stained with Masson's trichrome. The secretion was reddish-green in colour, in contrast with the normal grey or grey-green of normal secretion; the change was presumably due to disturbance of the secretory function. The regular "shattered" or cracked appearance of the contents of the acini suggested an insipissation of the secretion, which had become more brittle than normal after fixation (Fig. II).

In the epithelium there was an increase in the number of the laterally compressed, darkly-staining elements normally present, and on occasions these gave the appearance of having migrated and formed a layer between the "plug" of the secretion and the remaining epithelium. It was also often noted that the epithelium had become many-layered instead of comprising the normal single or at most two cell layers. In other situations the main elements had become cuboidal and not columnar, whilst occasionally there was almost complete erosion of the epithelium and a layer of the darkly-staining elements alone separated the secretion and the underlying connective tissues. The cells infiltrating the epithelium were usually lymphocytes, in spite of the numerous polymorphonuclear cells in the glandular lumina. The acidophilic globules appeared to be a feature of recently affected epithelium. They were frequently present where the cellular content of the secretion could be distinguished and an interstitial cellular infiltration had occurred, but were often absent where a caseous plug had formed. Changes in the interstitial tissues were relatively infrequent, but there was some increase in the number of connective tissue cells and fibres. Inflammatory elements were mainly lymphocytic and plasma cell in type, and again with a tendency to linear arrangement and formation of new fibrous tissue.

Ampullae

In all cases there were well-marked lesions which, as elsewhere,

were characterised by interstitial cellular infiltration, increased fibrous tissue and evidence of the disturbance of epithelial function, even where an abnormality was not easily detected in the epithelium itself.

In bulls Nos. 1 and 2 a feature of the interstitial change was the presence of well-defined nodules, which somewhat resembled lymphoid follicles (Fig. IV). They were spread throughout the duct from the tissues immediately beneath the epithelium to the part next to the muscle coat, but were found with greater frequency in the former situation. They were regularly oval or rounded and their centres often appeared to have retracted from the cells which surrounded them probably during the process of fixation. The cells of the centre were loosely arranged and were predominantly of a large polygonal type. They possessed round or oval nuclei containing scattered, coarse chromatin particles. In addition, there were large lymphocytes and macrophages, but only a few small lymphocytes. The peripheral layers of cells, which in effect appeared to be the same as those of the diffuse subepithelial infiltration, comprised darker staining elements, principally lymphocytes and plasma cells with various intermediate forms. These were usually more densely packed and concentrically arranged; moreover, collagen bundles with fibrocytes were found between them. The subepithelial infiltrations, which were generalised, consisted particularly of rows of lymphocytes and plasma cells (Fig. III). The latter possessed a fairly abundant, darkly-staining cytoplasm and a round nucleus often with chromatin arranged in "cart-wheel" form. Cells from these layers penetrated the epithelium, where lymphocytes, a few eosinophils, and even plasma cells could be found. Peripherally there was no cellular infiltration of the muscularis, but its inner layers showed an increased amount of intermuscular fibrous tissue. The interstial fibrous tissue so formed showed great thickening, especially centrally around the star-shaped lumen of the vas deferens. The cellular increase produced a compression of many of the glandular lumina, particularly those which lay peripherally. The epithelium of the glandular evaginations had become many-layered and showed numerous vacuolated cells, the vacuoles usually lying peripheral to the nucleus. Cells containing red globules, as described in the vesiculae, were more scanty. Cellular debris and, less often, a homogenous secretion were noted in the lumen. Amongst the cells were some rounded macrophages with abundant cytoplasm and a vesicular nucleus; they often included acidophilic globules or ingested lymphocytes. The remainder of the cells were granular leucocytes and lymphocytes together with epithelial debris. Occasionally the epithelium of the ducts showed in its superficial layers rounded, punched-out lacunae which were empty or contained several granular leucocytes (2 to 6). These patches probably represented areas where dead epithelium had been or

was being resorbed. This was most marked in bull No. 3 (Fig. III).

Vas deferens

This vas was affected in all animals though more severely and acutely in bull No. 2. In all cases there was a subepithelial infiltration of inflammatory cells. In the early stages of the lesion, they comprised eosinophils, plasma cells, lymphocytes and large mononuclears in varying proportions: later there was a formation of a fibro-cellular granulation tissue. The process extended as far as the muscle layer which was actually penetrated in bull No. 2. This change occasionally resulted in the formation of definite nodules which appeared to cause constriction of the lumen. The organisation of the submucosal exudate was sometimes perpendicular to the lumen at some places and parallel to it at others. The basement membrane was often obscured.

The epithelium in certain areas showed an irregularity varying from one to many cells in thickness. Small lymphocytes, polymorphonuclear leucocytes and occasional larger mononuclears were found, singly or in groups, between or sometimes within the epithelial cells. There was a distinct tendency for eosinophils to form dense clumps within small vacuole-like spaces, where presumably a cell had degenerated.

In bull No. 2 the surface of the epithelium was ragged showing groups of detaching cells. No noteworthy nuclear or cytoplasmic changes were seen; moreover there was no true ulceration, although desquamation may have proceeded to the stage where only one or two flattened layers of epithelium covered the lamina propria.

Epididymis

In all cases the changes were of such extent and of such long standing that it was impossible to speculate on the form of the primary lesion. The section of epididymis of bull No. 3 had the least advanced changes, and that of bull No. 2 revealed the oldest inflammatory process. In all cases the following were the main features. The tubules were dilated but contained few or no spermatozoa. Where present the content of the tubules consisted almost exclusively of a few eosinophils with some structureless cell debris and rarely a few recognisable spermatozoa. The epithelium which often consisted of a few superimposed layers of cuboidal or polygonal elements, was frequently invaded by eosinophils and lymphocytes, particularly the former.

In bull No. 3 the muscle coat of the tubules, which was intact and very well defined, was hypertrophied. In the other two animals the muscularis was somewhat obscured by the peritubular infiltrations and granulations. The distension probably caused the apparent thinning and irregularity of the muscle in these two cases. The lamina propria was consistently the site of a diffuse infiltration of chronic inflammatory cells, including large mononuclears, plasma cells and lymphocytes. Similar cellular accumu-

lations surrounded the muscularis in many fields and accounted for most of the cells to be found in areas of the interstitial granulation (Fig. V). It was commonly noted that the plasma cells in these situations were undergoing degeneration, evidenced by pyknosis and shrinkage of the nuclei and the accumulation of hyaline material within their cytoplasm (Russell body cells), especially in the large acidophilic globular masses. In material from all cases the age of the lesions was evidenced by the large increase of the interstitial fibrous tissue. In bull No. 2, the fibrosis was marked, but there were still present areas of fibrocellular granulation, including numerous sections of small blood vessels.

The most acute changes were seen in bull No. 1; these included an abscess 2 to 3 mm. in diameter, which probably arose from an infected and occluded tubule although all trace of the latter had disappeared (Fig. VI). The abscess consisted of a central zone with necrotic debris and very numerous polymorphonuclear leucocytes. Externally large mononuclear leucocytes and macrophages were prominent, whilst the outer edge consisted of lymphocytes and plasma cells with some tendency to fibrosis. In all animals it was possible to find clearly demarcated nodules resembling lymph follicles and identical with those seen in the ampullae. In some fields they appeared to be in close relation to the tubules, but this was not invariable. These nodules occurred less frequently than in the ampulla.

Testis

Histological examination of bull No. 1 revealed an extreme degeneration of the seminiferous epithelium. It was difficult to distinguish the individual tubules as their lumina were flattened and constricted to such an extent as to be hardly recognisable under the low power. They often contained masses of yellowish pigment, however, which facilitated their recognition. The "walls" of the seminiferous tubules consisted merely of an unlined connective tissue of which dense collagenous bundles constituted the greater part. No seminiferous cells remained. In between the collapsed tubules were irregular groups of intersitial cells; their relative frequency had, of course, increased, but no mitoses were visible and it was impossible to state whether there had been a hyperplasia. The rest of the interstitial tissue was of a dense fibrovascular structure; there appeared to have been an increase in small vessels but this might have been more apparent than real. There were very few inflammatory elements, although a few lymphocytes, plasma cells or Russell-body cells could occasionally be distinguished.

Bull No. 2 represented an intermediate stage. There was a very considerable decrease of spermatogenic cells. Only occasionally was the darker nucleus of a spermatogonium seen on the basement membrane. A few of these were in mitotic division. The tubular structure was intact but the lining was composed of

Sertoli cells; the nuclei of these formed a regular single layer just internal to the basement membrane. Their abundant lacy cytoplasm projected a considerable distance into the lumen and infrequently included a spermatogenic cell, sometimes in mitosis. The free border was somewhat discontinuous but fairly regular; in the smaller tubules the lumen was sometimes completely filled by irregular strands of cytoplasm and very occasionally a syncytium of Sertoli cells occupied the space where previously a tubule existed. If present, the lumen was devoid of anything but small amounts of structureless debris. There was evidence of a retraction of the tubules from the surrounding connective tissue; their diameter was certainly on an average less than normal and they were often surrounded by a clear space. The latter, of course, might have been a fixation artefact. In the interstitial tissue there was a definite increase in the number of connective tissue cells and fibres. Fibroblasts in various stages of differentiation were common. There did not appear to be any considerable accumulation of inflammatory elements, although a few small mononuclear cells were found scattered in some areas. Interstitial cells were not increased in number.

With the proviso that only one area of each testis was examined, it may be stated that the changes in this organ could be regarded as closely comparable to those produced by experimental or spontaneous hyperthermia—as in the cryptorchid. Nowhere was a significant inflammatory change observed; in addition, the observations recorded were in agreement with those of numerous authors who state that degeneration of the seminiferous epithelium is not accompanied by or followed by interstitial cell hypertrophy and hyperplasia. Any apparent increase could be regarded as a relative one depending on the atrophy of the tubules.

TABLE II

pH		*Fructose*	*B.C.*		*S.G.*	
No. of Specimens	*Mean*	*Mg/100ml semen*	*No. of Specimens*	*Mean*	*No. of Specimens*	*Mean*
6	7·80	0–200	5	3·15	4	1·0113
2	7·67	201–600	1	1·84	1	1·0294
4	7·34	601–680	4	2·10	4	1·0333
2	7·30	801	2	2·40	2	1·0276
8	7·41	201–800	7	2·15	7	1·0311

DISCUSSION

The data for the *pH*, buffering capacity, specific gravity and fructose content of the semen support previous findings that an alkaline *pH* is associated with a poorer buffering capacity, and a lower S.G., and a lower fructose content (Anderson, 1946, a and b; Anderson, 1951). It is of interest, however, to examine the present data in more detail to note departures from this generalisation.

An increase in the fructose content is accompanied by a decrease in the *pH* and an increase in the S.G., up to the level of 800 mg. fructose per 100 ml. semen, but the buffering capacity (B.C.) does not follow the other criteria. A fructose content below 200 mg. is accompanied by the highest *pH*, the poorest B.C. and the lowest S.G. With a fructose content of over 200 mg., the S.G. is normal, but the *pH* is high and the B.C. below normal. These biochemical characters of seminal fluid can thus vary independently of each other. On the present limited data normal values were obtained for frucose and S.G. associated with abnormal values for the *pH* and B.C. The normal B.C. and S.G. are 1·84 and 1·0377 respectively; an increase in the figure for B.C. denotes a poorer buffering capacity. (Anderson, 1946a.)

At post mortem the outstanding macroscopic changes were found in the epididymis and true testicular tissue. The former consisted of fibrous masses within which were entangled remnants of the tubules. Many of these had obviously become occluded with subsequent development of "lakes" containing muco-purulent material due to the obstruction of outflow of spermal fluids. The testicular tissue which was reduced in size, was pale and atrophic.

In general the histological findings agree with those briefly outlined by Hudson (1949). The character of the cellular exudate in this disease deserves comment in view of the probable elimination of some of the cells, at least those which reach the epithelia and lumina via the ejaculated semen. According to our observations these cells are very largely eosinophil leucocytes with a few small lymphocyctes, and the finding of such in appreciable numbers in the semen should be strong presumptive evidence of infection in bulls from herds known or suspected to be infected, even in early cases where no clinical abnormality of the genital tract is detectable.

The predominance of plasma cells and lymphocytes, especially the former, in the interstitial infiltrations agrees very closely with the description given by Christensen (1948) for Brucella infection of the ampulla and seminal vesicles of bulls. It is doubtful, therefore, whether the histology of the organs can be regarded as in any way pathognomonic for a specific venereal epididymitis. As noted by Christensen, it is interesting that the marked plasma cell proliferation is associated with local antibody production.

The pathogenesis of the disease requires a good deal of investigation. Its venereal transmission points to its being an ascending

infection of the genital tract; one would therefore expect that the seminal vesicles and ampullae would become infected first and, in fact, that some degree of establishment of the infective agent in these sites is a necessary prerequisite for infection of the epididymis. The frequency of localisation of Brucella in these organs in the bull, in cases where the genital tract is affected, has been noted by Blom and Christensen (1947).

The lesions in the accessory glands may perhaps heal largely by fibrosis, or become localised, whilst the epididymal lesion progresses. Histologically one could not say that the lesions of the accessory glands appeared older than those of the epididymis; however, the character and spread of any inflammatory reaction may differ considerably in the two organs.

The clinical condition of the epididymis is no guide to the composition of the seminal fluid, which is largely due to the seminal vesicles. This is illustrated by the difference between the bull with unilateral epididymitis (No. 3) and the two bulls with bilateral epididymitis (Nos. 1 and 2), although microscopically both epididymes from No. 3 were affected. It has been noted previously that a bull in which acute epididymitis had subsided, but which still showed clinical lesions, had a normal fructose content and an acid pH (Anderson, 1951). Judging from the physico-chemical composition of the semen throughout the period of observation, the condition of the seminal vesicles improved in Nos. 1 and 3 and deteriorated in No. 2, this being to some extent corroborated by the histological findings.

The pathological changes which were more marked in the seminal vesicles, vas deferens and epididymes of No. 2 than in Nos. 1 and 3 are in agreement with the poorer quality of the seminal fluid in this bull. Pathological changes associated with normal values for fructose, and S.G. and abnormal values for pH and B.C., were still evident in Nos. 1 and 3 at the time of slaughter.

The two latter estimations may, therefore, prove to be of greater value for detecting abnormal function of the accessory genital organs, particularly the seminal vesicles, than the former.

SUMMARY

An account is given of the pathological changes in the genital organs of bulls affected with a specific venereal infection, together with observations on the physico-chemical composition of semen from such bulls and the inter-relationship between the gland pathology and the seminal composition.

ACKNOWLEDGMENTS

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NOTE

Four photographs submitted by the authors and illustrating the macroscopic lesions of three affected bulls and one normal aged bull have been published by Professor M. W. Henning (1949) *Animal Diseases in South Africa* 2nd edition, pages 869 and 870.

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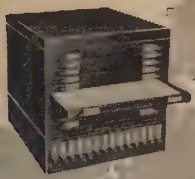

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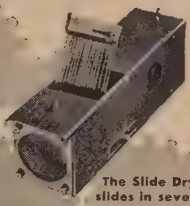

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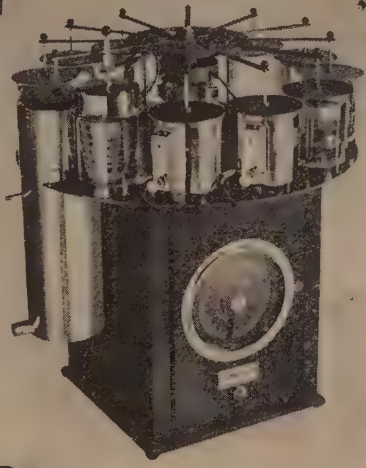

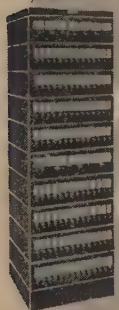

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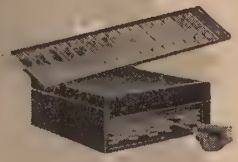

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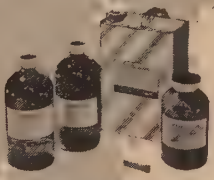

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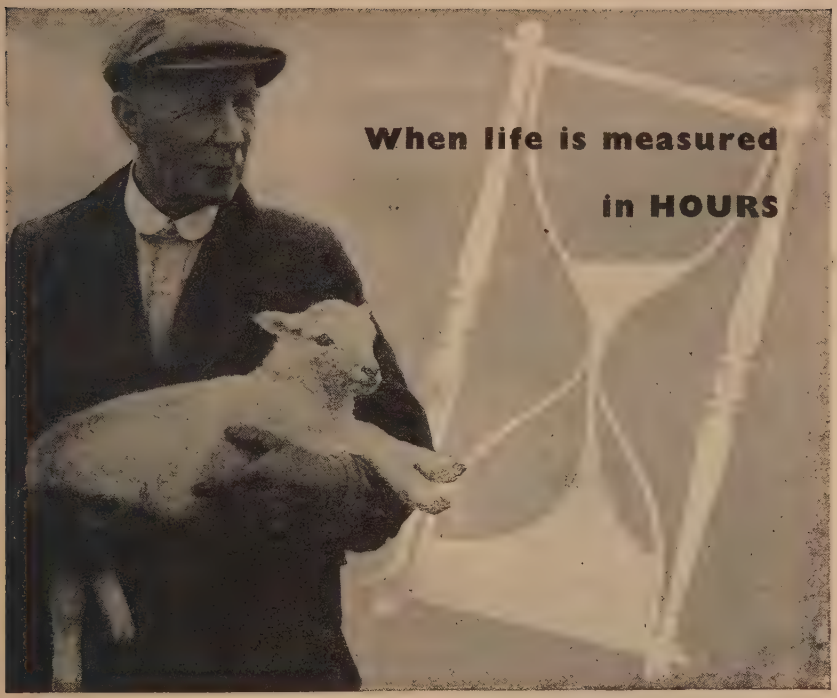

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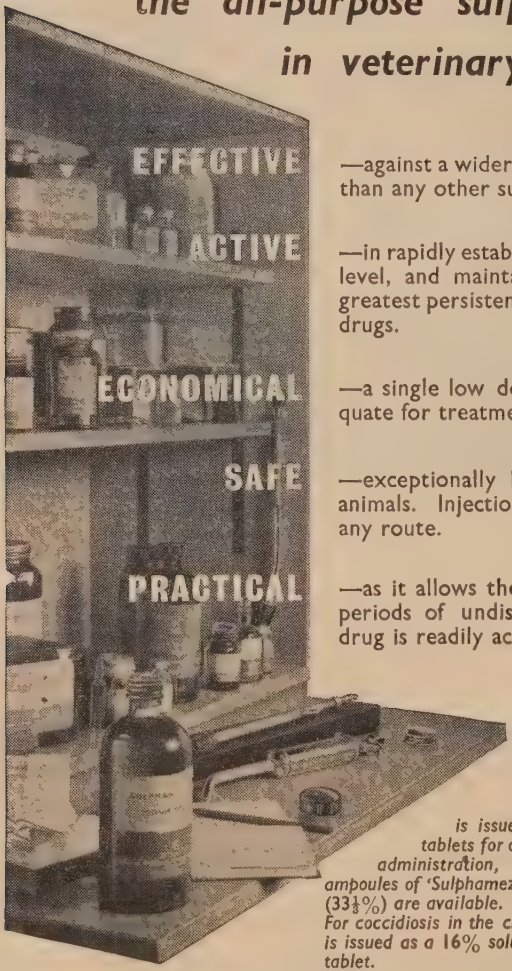

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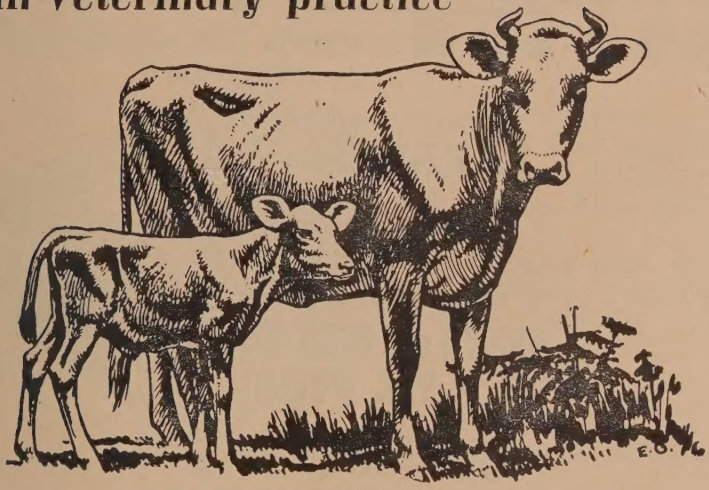

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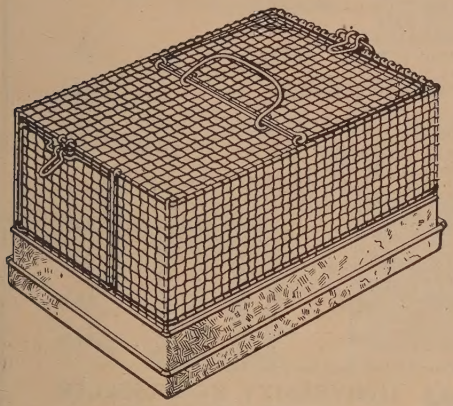

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